

Bias-free interracial comparisons

The danger that interracial comparisons will be inhibited by considerations of political correctness is less serious than that interracial studies will be wrongly used, yet much benefit could come from well-planned research.

DR Roger Bannister is a man of many parts. In 1954, he was the first person ever to run a mile in less than four minutes, since when he has become a medical neurologist of distinction. He is evidently also something of an iconoclast; last week he startled the British Association meeting at Newcastle upon Tyne by declaring that the prevalence of people (men and women) with dark skins on the starting-blocks and at the finishing-tapes of the world's athletics meetings is a sign that there must be something physiologically distinctive and advantageous in the constitution of dark-skinned people, otherwise 'blacks'. He added that the inhibitions decreed by political correctness should not prevent a dispassionate examination of such questions.

On the last point, Bannister is correct. He would also have been on solid ground if he had wrung his hands over the dangers there would be for science and for its successful application if there were serious grounds for believing that political correctness is, or will become, a significant obstacle to research. To be sure, some research is already restricted on the grounds of its social sensitivity. For example, many countries have followed Britain in requiring that research with human embryos should be carried out only under licence and should not extend for more than 14 days after fertilization. Few will describe those arrangements as a concession to political correctness. Rather, they are a necessary means of winning public confidence in the sober purpose of research in a novel and potentially subversive field. To judge from the annual lists of licensed research projects, curiosity is not yet being stifled.

Anthropology

To be fair, Bannister was not talking about embryology but about physical anthropology. That is an even more contentious field, and with good reason. Recent history echoes with claims to have established significant differences between racial groups in terms of mean IQ or mean cranial capacity. The late William T. Shockley of Stanford University (neither a physical anthropologist nor a psychologist) was a famous advocate of the dangers to society at large implied by those differences. To draw a fine distinction, Shockley was more a delayed echo of the naive eugenics movement of the 1920s than a racist in the mould of, say, Adolf Hitler. His arguments were none the less reprehensible on that account. He took as proven 'facts' whose provenance was at least dubious, and sought to found on them proposals for action by society at large whose consequences

would have been disastrous at least in the divisiveness they would have engendered.

Incentives

Bannister is no Shockley, but a decent British physician with a background in athletics calling attention to what seems to him to be a fact of life: the best athletes are black. But even the facts as they seem may not be facts in the strict sense. For example, it is well known that athletics is often the chosen pursuit of ambitious people from disadvantaged communities. Is not the legend of rags to riches in the prize-fighting ring or at the baseball diamond an established part of the American dream? Who can say how many of those who surprise Bannister at the starting-blocks at international athletics meetings have been impelled there by similar incentives? It is also likely that recent successes of black athletes from the United States have provided role models for black people from poor countries and, more particularly, have put into the minds of the governments of poor countries the notion that money spent on training athletes would enable them make a mark internationally.

None of this means that Bannister's hypothesis is self-evidently false, but that much more thought than he has given the matter would be required to devise, say, a successful research-grant application in the field. In such a context, the definition of 'black' would be a prior stumbling-block. The black people of Africa are believed to embody much greater genetic diversity than the rest of humanity put together; in the late Alan T. Wilson's classic study of human mitochondrial DNA, most clades in his cladogram represented black African populations. And nobody can guess what has happened in the melting-pots of the Caribbean and the United States to the genetic constitutions of those whose forebears were once slaves from Africa. Defining the study-sample for a well-founded research project would tax the ingenuity of the most prolific research-grant applicants.

There is also the matter of informed consent. The present convention (and sometimes the law) is that human subjects must not be engaged in research projects unless they are told in detail, and in advance, what it is hoped to accomplish, and how. That does not mean that interracial comparisons can be vetoed by anybody who cries out "Politically incorrect!", but that the research community must in prudence win the consent of the communities likely to be affected. Over the years, it has been amply demonstrated that patient explanation can win the consent of ethnic



groups to sensible research projects.

With physical anthropology under a cloud for its habit of using measurable skeletal indices as proxies for less tangible attributes (cranial capacity as a measure of intelligence, for example), it would be better to invest what goodwill there is in some quite different field. The Human Genome Diversity Project is already battling to win the consent of distinctive racial groups to schemes for collecting and analysing DNA. The goal is a better understanding of human evolution in the past 250,000 years or so. It is an important goal, from which all races will benefit. Such a project is unlikely to answer Bannister's question directly (nor can it say anything about mean IQ), but it could provide us all with a much better appreciation than we have at present of the wealth and interest of human diversity, and of the importance of the genetic constitution we have in common. □

New ways with wealth

The World Bank is devising a measure of national wealth that will be confusing if taken seriously.

NOTHING if not daring, the World Bank has devised a new measure of national wealth that is certain to capture wide attention because it relegates former top dogs to more lowly status in the international pecking order. For example, Australia comes out on top, Luxembourg and Switzerland are ahead of Japan, the United States is merely twelfth on the list, France stands higher than Germany and Britain is twenty-second, below Finland, Italy and the Netherlands. It is all good knock-about fun for the newspapers, but the bank's purpose is serious: to devise a numerical measure of national well-being that will be more appropriate to the assessment of sustainable development than old-fashioned gross national product (GNP) per head of population. The goal is laudable, but the method will set economists quarrelling among themselves (and with the bank) for months to come.

Environmentalists for many years have been fond of pouring scorn on GNP as a measure of anything that matters, and nobody denies that paradoxes abound. Suppose that a country is afflicted with an environmental problem, say a bad spill of radioactivity, and has to spend \$1 billion to clean up the mess. Evidently, the critics say, the quality of people's lives will have declined, yet the GNP will increase by a total of \$1 billion over the duration of the clean-up operation. In what sense, the complaint goes, can this needless expenditure be said to increase a nation's wealth? And can it really be accepted as true that the country would be worse off if it declined to clean up the mess, robbing its GNP of the \$1 billion in the process?

The reality is not like that, of course. GNP is not a measure of wealth, but of economic activity. Technically, it is an accident, although not a surprising one, that GNP per head is closely correlated with the sense of well-being that a country's residents enjoy. After all, on one definition, GNP is the aggregate of salaries and other payments to individuals. In any case, the World Bank proposes taking a radically

different line, defining the "wealth of nations" (it actually uses Adam Smith's term) in terms of asset values, tangible and otherwise. These come in four categories: natural capital (or the economic value of natural resources), "produced assets" (meaning the value of a country's built infrastructure), human resources (or the value represented by people's productive capacity) and social capital (described but not defined as the productive value of people collectively rather than individually).

At this stage, the World Bank's daring cannot be doubted. Economists will find plenty to quarrel about. Even the most tangible part of the bill of goods, called natural capital, cannot be estimated unambiguously. How, for example, is it possible to estimate the value of a buried asset except by the yardstick of a market price, no doubt abated by the estimated cost of extraction? And what happens when the prices of natural resources change relative to each other? Or when changing circumstances mean that a natural resource is no longer exploitable?

That is what has happened to Britain's coal reserves, described by W. Stanley Jevons a century ago as the foundation of Britain's then-prosperity, under the pressure of rising wages (a consequence of prosperity) and the prevailing 'not in my backyard' syndrome. By the same test, Jamaica does well in the new pecking order of wealth (at ninety-second) because its bauxite has been recognized as worthwhile within the past century (before which it had no value). Similarly, Papua New Guinea is listed above Latvia and Thailand on the strength of its recently discovered copper. The moral is that even the natural capital element in the new index of national wealth will have to be radically adjusted from time to time as the value of buried wealth changes. Will Qatar be eighth in the World Bank's list of the wealthy if thermonuclear fusion proves to be cheap?

Arguments about the valuation of human capital will be even more vigorous. Is this determined by the average cost of schooling, or by other criteria? In the estimation of the contribution to a country's wealth made by a motorway or a railway, should some account be taken of the degree to which the facility is used, or how it may be used in future? Counting the capacity of pin-heads to accommodate angels may be easier than answering these and related questions. But the valuation of social capital is an even more dangerous can of worms: who is to assess the relative value of a golf-club and parent-teacher association?

So far as can be told, the World Bank has set out to construct what businesspeople would call a balance sheet, not the single line called 'turnover' in a standard profit-and-loss account which is at present measured by GNP. (On present performance, 'social capital' is the equivalent of the intangible called 'goodwill'.) The World Bank's press release on the subject is misleading, suggesting that the new index is a *better* measure of wealth when it is merely a *different* measure. The whole question is to be discussed at length at a conference in Washington next month. The authors of this brave exercise deserve to have some well-wishers in the audience. In the interests of good sense, it is to be hoped that that there will also be some traditionalists. □

Pig heart transplant 'breakthrough' stirs debate over timing of trials

London. A British biotechnology company last week announced that it had achieved a major step towards the successful implantation of pig hearts into humans by breeding a transgenic pig whose organs can block the action of a key protein responsible for transplant rejection.

David White, co-founder and medical director of Imutran, based near Cambridge, told a press conference in London that 10 monkeys, each of which had been given a heart from one of the company's herd of transgenic pigs, had already survived for an average of 40 days, and that two were still alive more than 60 days after the operation.

The news that the company appears to have successfully developed a technique for combatting one of the major hurdles in xenotransplantation, the so-called 'hyperacute rejection' (HAR), has been widely welcomed among researchers in the field.

"It is a real step forward; I am very excited," says Fritz Bach, of the Harvard Medical School's Sandoz Centre for Immunobiology. Bach was one of the first to show that the failure of untreated organs to suppress the action of human complement is a major factor responsible for the rejection of donor organs.

There was a similar welcome to Imutran's news from transplant surgeons, currently squeezed between an increasing demand for transplants — in Britain, over 6,000 people

are currently waiting for organs to become available, and in the United States the number is more than 30,000 — and a declining supply of suitable organs from human donors.

Speaking at last week's meeting of the British Association in Newcastle, for example, Michael Thick, a local transplant surgeon, said that he and his colleagues were "waiting with bated breath for the case to be proven in clinical trials".

At the same time, however, opinion is divided among researchers over the speed with which the company says that it now intends to move on to clinical trials, given in particular the fact that its results have yet to be published in the scientific literature, and that other factors in addition to HAR play a role in organ rejection which is yet to be fully understood.

Imutran says that it believes its technology "is now ready to be tested in humans", and that it expects its first trial to begin next year in the United Kingdom, at the Papworth Hospital near Cambridge. John Wallwork, director of cardiac transplantation at Papworth Hospital — and also a co-founder of Imutran, set up in 1984 — says that the planned trials "will be a big step forward in the development of a genuine advance in human transplantation."

Smith says that the implications of Imu-

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REASONS

Imutran

Looking promising: two of Imutran's transgenic pigs.

tran's results for future xenotransplantation is that "HAR is no longer an issue". He adds: "The big debate now is, do we currently have the skills to keep the hearts functioning in people for a long time; and the only way to answer that question is to put them into people and find out."

Some researchers in the field argue that such experiments are premature. They claim in particular that more needs to be understood about other longer-term mechanisms in addition to HAR which can lead to eventual transplant rejection.

White, however, says that unexpectedly high survival levels of the monkeys given the transgenic hearts has boosted the company's confidence in moving forward. "A lot of people have said that [tackling the problem of HAR] was only the first of many hurdles," he says. "But as far as we can see, the other hurdles have not raised their head of the timeframe of our experiments."

Indeed, he is critical of those who, he claims, are holding back without warrant. "We are talking about designing a Model-T Ford, while other people are saying 'let's not go out driving until we have a Ferrari'."

Nevertheless, given the many uncertainties that remain, the optimistic tone surrounding last week's announcement has received some criticism for raising unrealistic expectations among those currently awaiting organ transplants, and encouraging the idea that human organ donors may be in less demand in the near future.

"One should not underestimate what Imutran has achieved," says Brian Pentecost, medical director of the British Heart Foundation. "At the same time, it is very important that the public does not come to believe that there is going to be a solution just around the corner, and that the need for individuals to volunteer as donors should not be lost."

White, however, firmly rejects charges that his company is guilty of raising unfair expectations — and in particular that last week's announcement via the press was ►

Astronomers keep an eye on the sky

Boston. Astronomers are pushing ahead with plans to search for Near-Earth Objects (NEOs), despite a decision last month by the US National Aeronautics and Space Administration (NASA) not to finance an expanded hunt for asteroids and comets on a potential collision course with Earth.

A 90-cm telescope at the Observatoire de la Cite d'Azur in Caussols, France, should become the next instrument to be used in this search. This will be followed by LONEOS, a new programme at the Lowell Observatory in the United States, and another survey that would make use of the US Air Force telescope network known as GEODSS (Ground-based Electro-Optical Deep Space Surveillance).

"We don't need to wait for a \$42-million programme," says Alain Maury of the Cite d'Azur observatory, referring to the recommendations of the so-called Shoemaker Report that were recently rejected by NASA. "We can do a good job for much less making use of the telescopes available to us now."

The French survey should begin before the end of the year, making it the second automated NEO search in the world, after the University of Arizona's Spacewatch programme at Kitt Peak. Initially, the Caussols telescope will be equipped with one charge-coupled device (CCD) detector, putting it roughly on a par with the Spacewatch telescope which discovers about between two and five NEOs per month.

Maury has already acquired nine CCD detectors and plans to have them all installed within a year, which would make his telescope by far the more powerful instrument.

Meanwhile, Maury has teamed up with astronomers at the German space agency, DLR, in an attempt to secure observing time at a 1-metre telescope at the European Southern Observatory in Chile. A Southern Hemisphere telescope would be a major advantage, as it would enable astronomers to find and track NEOs in both the northern and southern skies.

Steve Nadis

partly motivated, as some have suggested, by the need to raise more venture capital to support further experiments.

"We have made a point of stressing that there is no way that transgenic pig hearts are going to be generally available to people within less than five years," he says, adding that his company is receiving full financial backing from the pharmaceutical company Sandoz, and that "there is no public share offer in the offing".

The decision to make the results of Imutran's experiments available to the press prior to their publication in the scientific literature, he said, was based on the fact that some media stories were already appearing following a presentation to a recent conference in Amsterdam.

At the same time, White promises that the planned clinical trials at the Papworth Hospital will be carried out "under guidelines from the regulatory authorities". If such experiments do, in fact, begin next year, then such authorities will have to work swiftly; the British government, in a move which gave all the appearances of having been precipitated by the Imutran announcement, has only just agreed to set up an advisory committee to establish an ethical framework for xenotransplantation experiments.

According to an announcement by officials of the Department of Health, made unusually during the course of last week's Imutran press conference, the advisory committee will be chaired by Ian Kennedy, professor of medical law and ethics at Kings College in London.

White argues that there are no major ethical issues to be resolved concerning the principle of using animal organs for human transplantation, pointing out that pig insulin, for example, is already used to treat diabetes. More difficult, he admits, will be deciding the criteria on which patients will be selected for the experimental treatment, although even here, he feels, some patients may be prepared to accept highly experimental treatment if it is their only prospect of survival.

But the advisory committee can still expect strong reaction from animal welfare groups. Compassion for World Farming, for example, a group which has been actively engaged in issues such as the fight against the patent on the Harvard Oncomouse, has already expressed its opposition to xenotransplantation, suggesting that the solution lies increasing the number of potential human donors — as well as encouraging life-styles that reduce the need for organ transplantation in general.

Imutran officials say that they are remaining on their guard against possible protest action from animal welfare groups. They already have a herd of about 400 transgenic pigs, which are being kept in Cambridgeshire. But the precise location of the pigs is being held secret to prevent attacks from extreme animal rights groups.

David Dickson

Physicists warm to French strategy on nuclear tests

Paris. Seen in its broad political context, more good than harm is likely to result in the long run from France's decision to carry out a final series of nuclear tests in the South Pacific. This, at least, appears to have been one conclusion to emerge from a debate among physicists held in Paris last week on the role of scientists in the internal and public debate over nuclear weapons.

Richard Garwin, IBM Fellow Emeritus at the Thomas J. Watson Research Center in New York — and the main speaker at the meeting, organized by the French Physics Association — said that the planned series of tests is "not only acceptable, but desirable, if it leads to a zero-yield comprehensive test ban treaty".

Garwin, who was an adviser to the Los Alamos weapons laboratory between 1950 and 1993, and also to US presidents John F. Kennedy, Lyndon Johnson and Richard

both the United States and the United Kingdom to ignore parts of their own military establishments and make similar commitments to a "zero-yield" ban.

Previously, the five nuclear weapons state members of the United Nations Security Council had been pushing for a Comprehensive Test Ban Treaty that would have allowed nuclear tests with yields up to around 1,000 tonnes (see *Nature* 376, 283; 1995). This so-called 'threshold' posed a greater threat to non-proliferation efforts than the French nuclear tests, argues Hubert Reeves, an astrophysicist at the Centre National de la Recherche Scientifique.

Reeves, who attended last week's meeting, has been an outspoken opponent of France's decision to resume nuclear testing. But he now says that he is "less worried" than previously about the consequences of the French decision.

According to Reeves, Chirac's commitment to a zero-yield test ban has dispelled much of the distrust created among non-nuclear weapons states by his earlier announcement to resume testing. He describes the gathering momentum for a total test ban as "a happy ending", and points out that this was probably influenced by the scale of public protests at French tests. "Something good always comes out of something bad", he says.

The decision to invite Garwin to be the main speaker at last week's meeting reflects the fact that US scientists have ironically played a bigger role in the internal debate in France over the decision to resume nuclear testing than the country's own scientists.

Controversy was generated earlier this summer, for example, by a joint report written by members of the US National Resources Defense Council (NRDC) and the Federation of American Scientists (FAS) — of which Garwin is vice-president — disputing the French government's justification for the planned tests.

In particular, the report argued that there was a contradiction between French claims that the existing arsenal was reliable and that two tests were needed to maintain the stockpile against the effects of ageing.

The report stimulated a lively exchange in the French press between Garwin and Jacques Bouchard, the director of the Military Applications Division of the French Atomic Energy Commission.

French scientists have obtained more than 2,000 signatures for a petition denouncing the French tests. But Reeves and others agree they have had a lower profile in the debate than their US counterparts. "I said to my colleagues, where's the spirit of '68'?", says Reeves.

Declan Butler



Nixon, said he was unconvinced by the French case for resuming the tests, the first of which took place two weeks ago.

But he added that, if French officials are convinced that they need the planned series of tests, then their decision to proceed will be a small price to pay for French agreement to a total test ban. There was little dissent from most of the French physicists attending the meeting, even though many had previously expressed strong criticism of the tests.

Most public attention has focused in recent weeks on the resumption of testing. But many nuclear weapons experts feel that the long-term significance of this decision may be less than that of the recent commitment by Jacques Chirac, the French president, to support a ban on all nuclear tests, no matter how small, from next year (see *Nature* 376, 540; 1995).

Indeed, Garwin went so far as to describe Chirac's promise, if sincere, as giving France a "leadership" role in the non-proliferation arena. He said that it has already prompted

Clinton billed to back US research effort...

Washington. President Bill Clinton will speak out in support of his science and technology programmes at an awards presentation at the White House in mid-October, according to his science adviser, thus ending a long silence that had led many observers to question his commitment to the programmes.

Jack Gibbons, the science adviser, promised last week that the administration will defend its planned technology programmes "to the death". At the same time, however, he hinted that it is close to giving up hope of getting any new money for one of them, the Advanced Technology Program (ATP).

University representatives and science policy experts in Washington are concerned that, as the budget clash between the administration and the Congress approaches its climax, Clinton has publicly concentrated on defending education and some social programmes. They fear that he will fail to oppose Congress's proposed cuts in science and technology effectively.

The main cuts proposed by the Republican Congress so far are in the ATP, other assistance for industrial development, energy and transportation research, and science connected with global warming and the environment.

At a briefing with Laura Tyson, Clinton's economics adviser, Gibbons cited "Japan's

plans to double its investment in research" as the main reason for opposing the proposed cuts. Gibbons strongly attacked



All hands on deck: Clinton is scheduled to back the efforts of Gibbons (top left).

the cuts in ATP funding and in environmental research, including NASA's Mission to Planet Earth programme (see page 191).

Asked why Clinton has not publicly responded to criticisms of these proposed cuts, Gibbons said that the president "had a lot on his plate" at the moment. "We wouldn't be saying these things if we weren't

fully assured of the president's views," Gibbons added. "In mid-October he's planning a major address on science and technology issues." The address will take place at the presentation of the National Science and Technology Medals, an event always held at the White House, but only occasionally attended by the incumbent president.

Congress had been due to present its budget bills by 1 October, but is running a little late, so the address will come just as Clinton is entering the crucial phase of budget negotiations. Unsurprisingly, Gibbons and Tyson declined to say which programmes he will defend. "These bills are not going to end up as they are now," declared Tyson.

But Gibbons did suggest that the administration is likely to retreat on ATP, the most contentious single issue in the science and technology budget, for which Clinton has requested \$500 million and the House of Representatives has appropriated nothing.

He said the president would argue for "saving a portion of the ATP, and at least fulfilling the commitments that have already been made". His words left the clear impression that the White House is moving closer to the position of the Senate, which appropriated \$75 million to wrap up existing ATP projects. Colin Macilwain

... as NIH hopes for the best out of coming budget crunch

Washington. A US Senate committee has voted to give the National Institutes of Health (NIH) a \$300-million boost over this year's funding, equivalent to a growth of 2.7 per cent at a time when many other programmes are being heavily reduced.

But the committee's panel's efforts are overshadowed by a threat from President Bill Clinton to veto the broader bill in which the NIH budget is included and halt the entire budget process if Republicans do not begin negotiations over their proposed cuts in other social programmes.

One possible outcome could be the temporary closure of all government agencies — including the NIH — on 1 October, the beginning of the new fiscal year, if no agreement is reached by then. That could lead to delays in the receipt by NIH-funded scientists of next year's research grants; at present, 400 review groups are scheduled to meet in October to discuss such awards, which are usually made in December.

The bill approved by the Senate Appropriations Committee, and likely to be adopted by the full Senate later this week, would provide \$11.6 billion for NIH in fiscal year 1996. This represents a 2.7 per cent increase over the funds received by the biomedical

research agency in the current year, and contrasts with both the 5.7 per cent increase proposed by the House of Representatives and a 4.5 per cent increase proposed by the president in his initial budget request.

The House of Representatives and the Senate will work out the differences in their versions of the bill — which covers appropriations for the departments of Labor, Health and Human Services (HHS), and Education — in a conference committee.

If the committee agrees to a compromise figure of 4.2 per cent, this would be welcomed by the community as the best that could be expected in the circumstances. "That would be a very good number," says Dave Moore, of the American Association of Medical Colleges.

But what happens after the conference is anyone's guess. Clinton has threatened to veto the bill, not only because its proposed funding for education is too low, but because he feels that more money should be given to the whole budget for labour, education, health and other social programmes.

The Senate bill, drafted by the moderate Arlen Specter (Republican, Pennsylvania), chair of the Labor, HHS and Education appropriations subcommittee, is missing

most of the legislative directives that had been included in the House bill. These include a ban on embryo research funded by the NIH, and the elimination of separate funding for the Office of AIDS Research. But Bob Livingston (Republican, Louisiana), chair of the House Appropriations Committee, has said he will push to restore the restrictions in conference.

The Senate bill puts more emphasis on education and social services than the House bill, leaving less money for NIH. The National Cancer Institute, the National Center for Human Genome Research, the National Institute of Allergy and Infectious Diseases, the National Center for Research Resources and the Library of Medicine receive the largest increases from the Senate, in each case more than 4.6 per cent.

The Senate bill recommends \$1.38 billion for the Office of AIDS Research, a \$53-million increase over the 1995 level but \$19 million less than the president's request.

The NIH has asked that a few thousand of its 15,000 employees should be allowed to attend to patients, animals and cell lines in the event of a shutdown. Such an exception is normally made only for "essential" employees. **Adrienne Appel**

Patent office under fire over consultation

Munich. The European Patent Office (EPO) has come under fire from groups opposed to the patenting of genes and living organisms for declining to consult them over draft proposals, discussed at a forum in Munich last week, on how the European patent system could operate more effectively.

Four months ago, the EPO distributed to selected organizations a document drawn up by its administrative council, a body made up of representatives — usually the president of the national patent office — from each country that has signed the European Patent Convention.

The consultation document analysed, in particular, two major problems that the EPO faces. The main thrust concerned the high cost of European patents (see *Nature* 371, 371; 1994), but the public controversy over gene patenting was also put up for discussion.

But the EPO administrative council decided to restrict the hearing to groups concerned professionally with patents — primarily patent lawyers and representatives of industry. The European Commission, which is developing a new European Union patent, was also represented.

This move has stimulated widespread criticism from public interest groups concerned with patent issues. Sue Mayer, for example, a member of Greenpeace who has been actively engaged on topics such as the

patenting of the Harvard Oncomouse and herbicide-resistant plants, says that it was “foolish to restrict the debate, when the EPO should be trying to understand public concern [about patenting of life forms]”.

Mayer and others have been particularly critical of the EPO document for claiming that attitudes towards patenting medical processes have recently changed, and that “a majority of EPO users would be in favour of scrapping” the current exclusion from patentability of plant or animal varieties. This is enshrined in an ambiguous clause of the 1973 European Patent Convention.

David Shapiro, executive secretary of the London-based Nuffield Council on Bioethics, points out that this statement was presented with no supporting evidence — and appears to conflict with the general mood in the European Parliament in its vote opposing gene patents earlier this year (see *Nature* 374, 103; 1995). Shapiro and Meyer both claim that statements such as those in the EPO document — which was admittedly drawn up before the parliamentary vote — merely indicate that the EPO administrative council is out of touch with public opinion.

Shapiro says he heard about the document only indirectly, and was invited by telephone to attend the hearing only two working days before it opened. He accepts the appropriateness of inviting patent lawyers and innovation groups to the hear-

ing, which was primarily concerned with technical matters such as the high costs of patents. “But in matters of public interest it will not do to claim that you have had a public meeting when so many groups were excluded,” he says.

Gerald Weiss, who heads the secretariat of the administrative council, defends the council’s organization of the hearing, saying that the intention was primarily to gain input from user groups on ways in which patent costs could be reduced, and not to consider the patentability of biotechnology products.

He also says that the document was circulated among 250 journalists, though he admits that an invitation to attend the hearing was extended only to one journalist — Tom Wilkie, science editor of the *Independent* newspaper in London, who submitted a written response. But leading journalists in Germany say they have no recollection of having received the document, and that they would have been interested to attend the meeting if they had been invited.

The strong representation of national patent offices on the administrative council is felt by some to have made it impossible for the EPO to tackle another factor contributing to the high cost of patenting through the EPO, namely that half of the fees paid to renew patent protection in designated countries remains with the patent office in those countries (see *Nature* 371, 371; 1994).

Many EPO staff members consider this an unfair subsidy of national patent offices, to the detriment of the EPO. Yet the administrative council is unanimous that the formula for distributing the fees should not be changed.

The EPO’s apparent conservatism could be shaken up by the European Commission. The commission has been developing for a number of years progress on legislating for a community patent that would be valid in all 15 member states (which contrasts with the EPO patent which is valid only in countries designated by the patent holder).

Progress on this has until now been slow. Only seven member states have so far ratified the 1975 Community Patent Convention, and a diplomatic conference held in 1992 to find ways of speeding its enforcement was unsuccessful, primarily because of fears that the high costs involved in providing translations of applications into all EU languages would render the community patent unused.

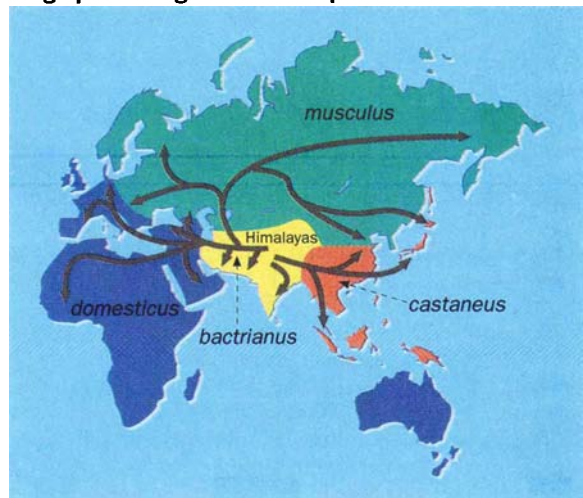
Also, the commission is coming to the end of a major consultative phase on its failed directive on patenting of biotechnological products and will decide within the next few weeks if a revision will be put to the European Union Council of Ministers and the European Parliament **Alison Abbott**

How the mouse's tale began life in India

New Delhi. Genetic studies of wild Indian mice have led to a discovery that the house mouse, *Mus musculus*, originated in northern India. The finding has raised for the first time the prospect of inbred Indian strains being used as models by biologists.

A special animal facility at the National Institute of Immunology (NII) in New Delhi has established a colony of wild mice collected from 30 different places in India for breeding the strains. Pairs of mice have been inbred down to 14 generations. According to Rakesh Anand, who heads the facility, researchers “will be ready to supply these strains to research laboratories” after six more generations.

The current stocks of mice in laboratories worldwide are all derived from three subspecies: *Mus musculus domesticus*; plus *musculus musculus* and *Mus musculus castaneus*. A study carried out jointly by NII and the Institut Pasteur in Paris has now established that all three are offshoots of the original house mouse, which began migrating from India 900,000 years ago (see diagram). K.S.J.



New IPCC report set to confirm earlier warming conclusions

London. The principal United Nations advisory committee on the scientific nature and impact of climate change is expected to confirm its initial assessment, first published five years ago, that human activity is playing a significant role in global warming.

Despite continued scepticism from some scientists, the new report, due to be published later this year by the Intergovernmental Panel on Climate Change (IPCC), is expected to repeat earlier warnings made in its first report, published in 1990.

A draft version of the "second assessment synthesis report", which has been circulating for comment in parts of the scientific community, says that observed increases in the global mean temperature of between 0.3 and 0.6 °C "is unlikely to be entirely due to natural causes".

Comparison of observed temperature change with model simulations, says the draft, means that "the best evidence to date" suggests that "a pattern of climatic response to human activities is identifiable in the climate record".

Much of the new certainty comes from the increasing sophistication of climate models over the past few years, and in particular from results of running models simulating the combined effects of greenhouse gases (which tend to increase surface temperature) and shorter-term effects of aerosols (which tend to reduce it).

The report admits that the observed warming of the globe has been smaller than that projected by models that include the impact of greenhouse gases alone. It also admits that there remains considerable "unexplained variation" between the detailed patterns of the warming observed and model projections.

Nevertheless, in an important conclusion — which reflects that reached by its Working Group 1 and reported earlier this year — the draft report states that "agreement is generally better than those which include greenhouse gases alone".

IPCC officials are embarrassed that the draft of the second assessment report was 'leaked' through the Internet as part of its review process. They are quick to emphasize that the detailed conclusions may be modified in the final version of the report.

But scientists familiar with the debate that has been taking place on the content of the final report say that the broad thrust of such conclusions — in particular, that recent work with climate models has reinforced the conclusion that human activity is a major factor in global warming — is unlikely to be altered. □

BA puts government science move under the microscope

Newcastle-upon-Tyne, UK. Basic science in Britain appears to have more supporters in industry than in government, according to the evidence of a former science adviser to the UK Department of Trade and Industry (DTI) during the first years of the administration of the Prime Minister, John Major.

Addressing a public forum during the annual meeting of the British Association for the Advancement of Science, Geoffrey Robinson, director of IBM's Hursley Laboratory, said that industry is keen to conserve Britain's reservoir of basic science.

Science, on the other hand, is important to government, as witnessed by the amount of money spent on research. But, he added that government has no "real feeling for basic science," as it is not a high-profile, mainstream political issue. Governments, he says, fund science because of its prior commitment to the public funding of research.

"Many scientific issues are central to government policy, but this does not include the broad span of science. Sitting round the cabinet table talking of science in the abstract is not a principle of central government."

Robinson was a key figure in drafting the DTI's contribution to the 1993 science white paper *Realising Our Potential*. He left the government in January 1994 when his post was abolished during a reorganization of the DTI following both the publication of the white paper and a separate series of decisions to cut back the DTI's funding for technology research programmes.

But he still defended the government's moves to bring science closer to industry — including the recent controversial decision to move the Office of Science and Technology from the Cabinet Office into the DTI (see *Nature* 376, 103; 1995) — claiming that the move need not necessarily undermine basic science.

However, Robinson added: "I do think there is still only limited debate between industry and government on the importance of basic science."

"The notion that industry is competing with science is absurd," said Robinson. "Science and industry are pursuing two different but complementary agendas. A strong basic science is vital to industry."

Robinson said particle physics, which is relatively unknown to industry, ought to be held in high regard. "High-energy physics is a demanding and rigorous discipline which attracts many intelligent people," he said. "Industry needs first-class minds and particle physicists are first-class minds."

The forum was convened by Sir Martin Rees, Britain's astronomer royal and this year's president of the BA, to discuss the implications of the OST's transfer. The

move, of which there was no prior warning, has cast a dark cloud over three years of relatively good relations between government and scientists.

Many critics have claimed that shifting the OST to the DTI threatens curiosity-driven research, and that it has weakened the potential influence of both the government's new chief scientist, Robert May, who will now operate from within the DTI, and the highly regarded Ian Taylor, the new junior minister for science and technology.

Both May and Taylor were present at the Newcastle meeting and used the occasion to allay the concern of scientists. At the same time, however, both reiterated the government's belief that science must be better harnessed to the task of wealth creation.

Addressing a press conference, Taylor dismissed the government's critics as a small, if vocal, minority. "Most of the people I've talked to are positive about it. I've had a fistful of letters. They all realize that the future of basic science rests on a parallel development of that science in industry."

May suggested the importance of the OST as a relatively free-standing body within the Cabinet Office had been exaggerated, and added he could make an equally good case for other arrangements. "The problem is not where to place the OST, but to better translate our world-class research into wealth creation," said May. "In that sense, the DTI is a sensible place to put it."

May pointed out that the United Kingdom, while leading many other countries in the production of scientists and engineers, lags behind in ensuring their adequate representation in the workforce.

But dissenting voices still abound. Sir Sam Edwards, professor of physics at the Cavendish Laboratory, University of Cambridge, questioned both whether basic research can be usefully directed to enhancing the quality of life, and the wisdom of forcing scientists and industry to interact, when countries such as Japan without such a tradition appear more successful at wealth creation than the United Kingdom.

"We are the envy of the world in terms of interaction of universities and industry," said Edwards, a one-time chairman of the former Science and Engineering Research Council. "Many graduates come from overseas. If we're so awful, why do they keep coming?"

Indeed, Edwards put forward the unfashionable hypothesis that the United Kingdom has too many scientists — rather than too few — and that this is the main reason that salaries for scientists are lower than in other comparable professions. "This is an immutable law of supply and demand," he said

Ehsan Masood

Congress backs home for orphaned agencies

Washington. The Science Committee of the US House of Representatives has suggested that, if the Department of Commerce is abolished — as now seems likely — a new, independent agency should be set up to absorb the National Oceanic and Atmospheric Administration (NOAA) and the National Institute of Standards and Technology (NIST).

Proposals for such an agency, to be called the US Science and Technology Administration, were incorporated in a bill passed by the committee last week. They replace an earlier suggestion by new House Republicans led by Dick Chrysler (Michigan) to sell off the NOAA and NIST research laboratories, which absorb most of the two-thirds of the commerce department's \$4-billion annual budget spent on research, after the department's closure.

But the move was immediately criticized by Democrats as little more than a cosmetic

reorganization aimed at winning credit for abolishing a governmental department without actually changing the federal government's role. "There is no evidence that abolishing the Department of Commerce, and creating this new bureaucratic superstructure, will save any money," says George Brown (Democrat, California), the senior minority member on the science committee and a strong critic of Republican plans.

Brown's interpretation gained credibility when Robert Walker (Republican, Pennsylvania), chairman of the committee, unexpectedly accepted an amendment removing from the bill a provision that would have capped spending on all functions of the Department of Commerce at 75 per cent of their 1994 levels. The successful amendment was put forward by Connie Morella (Republican, Maryland), who is keen to protect the main NIST laboratory at Gaithersburg, which is in her congressional district.

But the idea of a new agency embracing the science functions of NOAA and NIST — a watered-down version, in effect, of Walker's earlier proposal for a far grander Department of Science (see *Nature* 374, 201; 1995) — is likely to survive when the House passes a bill to dismantle the commerce department.

The bill was approved by Walker's committee last week, after it had heard testimony on the future of the department earlier in the week from Ronald Brown, the commerce secretary, Barbara Franklin, his Republican predecessor, Chrysler, and 16 other witnesses.

Chrysler appeared to win few converts for his original proposal. He described the department as a "hall closet" — or cupboard — for the various bits and pieces which other government departments did not want. When asked by Vernon Ehlers (Republican, Michigan) whether he had such a cupboard at home, Chrysler replied, rather lamely, that he did, but that he also balanced his budget.

Calling for the privatization of the National Weather Service, Chrysler pointed out that "75 to 80 per cent of newspapers" already obtained their weather information from private sector sources. But committee members asked if he knew where the private sector obtained its data, underlining Chrysler's apparent lack of awareness that it is all provided by the weather service and is therefore supported by the government.

Brown clashed angrily with Franklin over a statement in her testimony that NIST had been "politicized" under its current director, Arati Prabhakar, and her claim that the way in which the Advanced Technology Programme (ATP) was now run "was the biggest part of that".

Challenged to produce specific evidence, Franklin, who supported ATP under the Bush administration, said she did not know what had been happening since she left office. "You don't like it because you're not in control of it any more," stormed Brown. He pointed out that Prabhakar was a career official, appointed on the basis of her impressive track record at ARPA, and admonished Franklin for "coming here to sloganize".

Three prominent Republican members — Morella, Sherwood Boehlert (New York) and Tom Davis (Virginia) — also supported a rescue of the ATP. Nevertheless, the bid was roundly defeated by the committee.

Other House committees are now considering the Department of Commerce Dismantling Act, which is likely either to be passed on its own by the House, or to be incorporated into a large 'reconciliation' bill at some point in the next few weeks (see *Nature* 377, 93; 1995). **Colin Macilwain**

Soros fund wins corporate support

Moscow. For the first time since the fall of the communist regime, a prominent business organization has promised to provide substantial support for Russia's basic research.

At a press conference held in Moscow last week, Boris Saltykov, the minister of science and technology policy, announced that the Joint Stock Company LOGOVAZ is to make a grant of US\$1.5 million to the International Science Foundation (ISF), originally set up with financing from the Hungarian-born financier George Soros.

The new money will be used to extend into next year the ISF's Conference Travel Grants Program, which supports the participation of Russian researchers at international conferences. Saltykov's announcement was made in the presence of both Soros himself and of Boris Berezovsky, the president of LOGOVAZ.

Although the amount involved is not large, its main significance is the fact that it is the first time a Russian businessman has joined efforts to support Russian science, thus re-establishing a strong tradition of philanthropy that existed in pre-revolutionary Russia.

At the same time, Soros himself has said that he intends to continue financing the ISF programme which provides Russia's scientific community with access to leading Western science journals.

Meanwhile, the Presidium of the Russian Academy of Sciences (RAS) has devoted its first meeting of the new academic year to a discussion of the proposed law on science and the state scientific and technological policy which is currently before the State Duma, the

Russian parliament.

The academicians are opposed to the definition of the status of the academy proposed in the new bill, namely that it should be classified as a "state organization", meaning not only that it will be financed from the state budget, but also that the state, for this reason, has the right to control its activities, in particular by nominating its officials.

Members of the academy say that they do not object to the principle of state financing of their activities. But they want the academy to be a 'self-regulating' public organization, and to be able to elect its leaders and distribute the money it gets from the state as it wishes.

Vladimir Kudryavtsev, vice-president of the academy and a lawyer by profession, suggested that a new status should be created for the academy as a "state-public" body. This would have the advantage of being financed by the state while avoiding the unpleasant consequences that could follow.

Until now, the Russian Academy of Science has seldom failed in its struggle against the government to retain control of its activities, and many observers feel that it stands a good chance of winning the current dispute.

In a separate move, the Russian government has issued a decree stating that the financing of the Russian Humanities Scientific Fund (RHSF) is to be doubled, and that in future the fund will receive 1.0 per cent of the total budget allocated for scientific research every year, rather than the former humanities allocation of 0.5 per cent. **Carl Levitin**

Space station funding dispute may delay ESA ministers meeting

Munich. The European Space Agency (ESA) may have to postpone its next ministerial meeting, scheduled to take place in Toulouse in mid-October, because member states are still unable to decide how to pay for their proposed contribution to the international space station.

But the content of ESA's proposed contribution at least has now become firm. Last week, France told a high level meeting of ESA delegates in Paris that it would withdraw the proposal it made in July to substitute the planned space laboratory, to be built primarily by Germany and Italy, with a French-built crew rescue vehicle (CRV) (see *Nature* 376, 286; 1995).

The proposal was drawn up in the light of French dissatisfaction with ESA's proposal earlier in the year to drop the CRV from ESA's three-element package. It threatened to delay a final decision on ESA's participation in the space station.

But France's withdrawal, precipitated by ESA's reluctance to change the balance of the programme at short notice, may substitute one headache for another. Because its industrial involvement has been curtailed by the dropping of the CRV, France may now be unwilling to put up the ECU600 million (US\$732 million) that it had previously agreed as its share of the ECU1.42-billion package, which runs from 1996 to 2000.

Italy, which promised ECU300 million, may review its level of participation, because of serious overcommitments in its space activities. In particular, its commitments to the space station through ESA, and through bilateral agreements with the US National Aeronautics and Space Administration, would absorb well over half of the total budget of its space agency ASI up to 2000.

The Italian government's budget plan for 1996 will not be unveiled until the end of this month at the earliest, and it is not known if it will include extra cash to rescue the space programmes. Furthermore, even if extra cash does indeed emerge, it is unlikely to be sufficient. Italian officials are believed to be advising Giorgio Salvini, the research minister, to seek to delay ESA's long-planned ministerial meeting until its own financial situation is clearer, and to renegotiate Italy's level of participation in the space station programme.

Of the three major participants, only Germany is certain to stick to its agreement to provide ECU300 million. Smaller participating countries are to provide ECU150 million. Even without the financial uncertainties of France and Italy, there still remains a shortfall on the total bill of more than ECU100 million, which has so far found no sponsors.

Alison Abbott

Earth observation programme 'needs tighter coordination'

Washington. The ambitious US Global Change Research Program (USGCRP), which seeks to use satellite data to develop understanding of terrestrial processes, was taken to task last week by a panel of the National Research Council (NRC).

The panel said in a long-awaited report that the multi-agency effort suffers from a lack of coordination that hampers its scientific output. At the same time, however, it concluded that the programme's research goals were fundamentally sound — a vote of confidence that should help it to survive the congressional budget axe over the next few weeks.

Earlier this year, Robert Walker (Republican, Pennsylvania), the chairman of the House of Representatives Science Committee, asked the NRC to review the USGCRP, which has requested \$1.8 billion in funding for the next fiscal year, to be distributed among eleven federal agencies that conduct environmental research.

Walker asked the NRC, part of the National Academy of Sciences, to pay particular attention to the National Aeronautics and Space Administration's Mission to Planet Earth (MTPE). This is the largest single element in the global change programme, and has been targeted by Republican lawmakers for deep cuts.

In its report, the NRC affirms that the USGCRP's "fundamental basis is scientifically sound". That finding should boost its credibility with Republican critics, who have accused the Clinton administration of manipulating the programme to support its agenda for setting up international protocols to counter global warming.

According to one panel member, Edward Frieman of the Scripps Institution of Oceanography, the committee found that the "science being done in this programme is of extraordinarily high quality, and is not being politically driven". Frieman says that Walker seemed satisfied with that answer when panel members briefed him last week.

The NRC panel calls for an "adequate and stable level of funding" for global change research. But it also criticizes the programme's lack of interagency coordination, claiming that this leads to inefficiency and missed science opportunities.

Such coordination is supposed to take place in the White House's National Science and Technology Council and its Committee on Environment and Natural Resources, widely touted as a "virtual agency" for government-wide environmental research. But according to the NRC panel, the virtual agency approach is not working.

Part of the problem is excessive focus on

individual agency budgets and not enough on presenting a coordinated, multi-agency research plan to which both the Congress and the White House Office of Management and Budget can agree. Frieman, cites a relatively obscure, \$4-million project funded by the DoE to determine the uptake of carbon by the oceans, which Congress has marked for elimination next year. "Here's a critical piece of the science which can vanish overnight because of the vagaries of one

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Flying high: one of NASA's planned satellites

particular agency's budget," he says. As for NASA's Mission to Planet Earth and its centrepiece, the Earth Observing System (EOS) of environmental satellites, the NRC committee gave another qualified endorsement. The panel called for the first two EOS spacecraft, AM-1 and PM-1, to go ahead as planned, and for a third, Chem-1, to undergo only minor modifications.

After that, however, the programme could be streamlined by taking advantage of new spacecraft technology, as Republican lawmakers and NASA's own study teams have already recommended. Most radical of all, the panel would give the EOS Data and Information System (EOSDIS) a complete overhaul.

The job of producing data for the scientific community would go to a competitively selected federation of government, academic, and industry partners, instead of the handful of government centres now foreseen. Privatizing the data distribution would be "enormously more effective and much cheaper", says Frieman.

Initial reaction to the NRC report in Congress has been favourable, with both sides of the funding debate claiming that it supports their position. The day before the report came out, the Senate subcommittee that oversees NASA's budget granted nearly the full amount for MTPE — cutting only \$61 million from a \$1.34 billion request, compared to a \$339 million cut that has been proposed by the House.

Tony Reichhardt

Restoring good manners

SIR — John Maddox asks how good manners can be restored to science (*Nature* 376, 113; 1995). He suggests that editors should act as better custodians of scientific virtue and that institutions and grant-making agencies also have responsibility in this arena. But the real problem is that science is bad-mannered by its nature. All of us want to be first, and push and shove to get there. And, should someone beat us to it, we delight in showing that they were wrong in detail if not necessarily in fact. This is healthy. Good, lasting, verifiable and useful science is made by the aggressive promulgation of ideas honed by the also-rans. No runners, no race.

But all is not so simple and Maddox is right to complain. Scientists do seem to be misbehaving to the extent that only a proportion of the vast literature now seems worth reading. And Maddox is largely correct in his targets. It is, however, journals and grant-makers that are principally contributing to the degradation of science. Vanity co-publication is a nuisance but is used by only a tiny minority to camouflage outright fraud. The total collapse of peer review within journals and by grant-making organizations is a far more serious threat to our common pursuit. Despite the fact that there are arguably more scientists extant than ever before, journals and grant-making bodies appear to ask ever fewer individuals to review their material for them. Their excuse may be inundation but the reason is laziness.

The journal I co-edit has a policy of varying its reviewers on the grounds that peer review should be regarded as little more than a market-research exercise, as I have mentioned in these pages and elsewhere before (*Nature* 364, 183; 1993 & *Redox Report* 1, 1–2; 1994). This is more work for us but ensures that we get a wide range of opinion, which we then evaluate. By contrast, it must be the experience of many senior scientists that they are constantly landed with submitted articles or grant applications on a particular theme. “Old Joel understands this stuff. Send it to him” seems to be the attitude. But this means that O.J. effectively controls the field. And O.J. may have gone bonkers in the decade since he last held a pipette or be simply allergic to certain schools of thought so the field stagnates.

Entrusting the right to authorship or allocation of funds to a few individuals also, of course, contributes heavily to feelings of unfairness and suspicions of patronage. The characters are too easily

identifiable and their prejudices too well known. The simplest way to identify the range of scientists capable of reviewing an application or paper is to use electronic search methods combined with a letter, fax, e-mail or telephone call. Yes, this does involve more work and potential delay. Yes, this may mean transcribing incomprehensible foreign addresses with lots of consonants run together. Yes, this may mean asking the opinions of researchers who do not have English as their first language and who “don’t know our system”, but the results, and benefit to science, would make it worthwhile.

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Benefits of dumping?

SIR — As a microbiologist, I rejoiced at E. G. Nisbet’s and C. M. R. Fowler’s Commentary on the beneficial effects of dumping the Brent Spar oil storage platform to the deep-sea microbial flora and can only share their disappointment that it was eventually cancelled (*Nature* 375, 715; 1995). As the father of two children, I also know too well what these microbes must have felt about their missing Christmas gifts (*Nature* 375, 708; 1995). However, life goes on, and there are plenty of gifts for which we should be grateful.

We should welcome controlled and accidental release of nuclear fission products in the oceans and elsewhere which, by their mutagenic virtues, provide our microbial friends with a rich spectrum of adaptive perspectives. Similarly, sites of waste disposal are populated by myriads of rapidly evolving microbial species just waiting for tomorrow’s new plastics. Wouldn’t it be heartless not to give them what they need? On the basis of the arguments published in *Nature*, a solemn proposal should be issued by the scientific community to the Austrian, Swiss and German governments to dump a number of scrap cars in Lake Konstanz (which is also full of microbes but more easily accessible to the average microbiologist than the deep sea). I am ready to join Shell Oil in its ecological efforts and would generously donate my old Volkswagen for this purpose.

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Fair’s fair

SIR — Michael Lardelli (*Nature* 376, 123; 1995) expresses concern about a rule of the Human Frontier Science Program (HFSP) that the principal applicant for a research grant “must be a national of one of the eligible countries”. Thus nationals of non-eligible countries, even if their laboratory is established in an eligible country, cannot be principal applicants.

HFSP was created on the initiative of the Japanese government in the framework of the Economic Summit; the eligible member countries are Canada, France, Germany, Italy, Japan, the United Kingdom, the United States, the other countries of the European Union and Switzerland. These countries fund the programme and the eligibility rule has been adopted as a fair requirement by the funding governments.

It should be emphasized, however, that this eligibility requirement applies only to the principal applicants, and that all other co-applicants can be of any nationality, eligible or not, provided that their laboratories are in two or more different countries. The purpose of the grants is to promote international collaboration. It is hardly restrictive when only one of the applicants must be from one of the 19 eligible countries.

Michel Cuénod

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Rampant paranoia

SIR — Robert Shields claims (*Nature* 374, 683; 1995) that one can “look in any journal of plant physiology [and find that *Arabidopsis*] hardly rates a mention”. This is an absurd statement; the mentioning of *Arabidopsis* in *Plant Physiology* — certainly a typical “journal of plant physiology” — is in fact excessive. The index to volume 103 (September–December 1993, one issue per month; the most recent volume in my office) lists 17 articles about *Arabidopsis thaliana*, and other articles in that volume also discuss *A. thaliana*. But it gets worse: one of the four issue covers of volume 103 of *Plant Physiology* highlights *Arabidopsis*. And, one of the other issue covers compares a tobacco gene to a gene of, that’s right, *Arabidopsis*. To be fair, I checked the index to volume 102 (May–August 1993) and found, once again, 17 entries under *A. thaliana*.

Shields is clearly suffering from “they don’t talk about *Arabidopsis* enough” paranoia, and you are helping to spread the disorder.

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Letters submitted for Correspondence should be typed, double-spaced, on one side of the paper only.

Natural antidote to global warming?

The condition of the Sun in the seventeenth century suggests that reduced radiation could have accounted for the Little Ice Age, but there is only a small chance that a recurrence will head off global warming.

COULD an engineered return of the Maunder minimum be a credible antidote to the threat of global warming in coming decades? Speculations of that kind naturally have no place in the real world of practical politics, with its carbon taxes and so on, but they are more substantial than mere nostalgia. What is the chance that the Sun will save our bacon by lapsing into a condition free from sunspots? And would that be enough to offset the effects of greenhouse gases in the atmosphere?

The Maunder minimum is the period from 1645 to 1715, close on the heels of Galileo's first observation of apparently black spots on the surface of the Sun. Throughout the Maunder minimum, there were no spots or other signs of surface activity on the Sun. Famously, the Maunder minimum came towards the end of what was known in the Northern Hemisphere as the Little Ice Age (spanning four centuries from 1450). In London, the surface of the Thames used regularly to freeze in the winter, encouraging novel forms of party-going. European literature of the period is replete with images of intense winter cold.

How would the lack of sunspots (and of other forms of activity) have affected the climate on the surface of the Earth? If there were a better understanding of what happens in the outer layers of the Sun, the answer might tumble out from a calculation of some kind, but that is not possible. But old-fashioned inference can accomplish a great deal. Ingeniously, J. L. Lean from the US Naval Research Laboratory and O. R. White and A. Skumanich from the National Center for Atmospheric Research at Boulder, Colorado, have now used a variety of proxies for solar activity to estimate both the output of energy from the Sun and its spectral distribution during the Maunder minimum (*Glob. biogeochem. Cycles* **9**, 171–182; 1995).

The chain of inference is elaborate, but all the more interesting on that account. How can one estimate sunspot numbers at times before people knew there were such things? The rate of production of cosmogenic radioactive isotopes, such as ^{14}C and ^{10}Be , by the interaction of cosmic rays with atomic nuclei in the upper atmosphere is one good proxy, because the intensity of cosmic rays reaching the Earth is modulated by the activity of the Sun. And how is it possible to infer the output of ultraviolet radiation from the Sun during

the Maunder minimum when accurate records of ultraviolet radiation (based on satellite measurements) are available only since about 1980? Use observations of other stars like the Sun as a guide, of course.

The isotope data were first decisively used by J. A. Eddy (*Science* **192**, 1189–1202; 1976). The essence of the technique is that the Earth is partially shielded from (comparatively) low-energy cosmic rays by an active Sun, with the result that cosmogenic isotope production is then decreased. The result is that the Maunder minimum is recognizable by extra ^{12}C in tree-rings and extra ^{10}Be in ice-cores from the period. Lean and his colleagues echo Eddy's original ambition that a careful comparison of past isotope production would allow a detailed reconstruction of the Sun's activity in the past, but evidently the data are not yet good enough to do more than identify a rather longer minimum beginning in the fourteenth century.

The other leg of the chain of inference is the use of contemporary records of ultraviolet emission from the Sun and observations of stars similar in mass and age to the Sun to reconstruct the ultraviolet spectrum of the Sun during the Maunder minimum. The crucial data are those due to Salie Baliunas and Robert Jastrow (*Nature* **348**, 520–523; 1990), who hunted through Palomar and Mount Wilson plates to find a sample of stars like the Sun and for evidence of their ultraviolet emission in bands due to hydrogen and potassium, taken as proxies for magnetic activity. Their conclusion was that some stars have magnetic cycles like the Sun, and that others do not.

The objective, as stated, is to estimate the ultraviolet output of the Sun during the Maunder minimum. Lean *et al.* first show that there is a tight correlation between the Lyman- α emission line, the He 1083-nm emission lines and the Mg^+ absorption line with the Ca^+ absorption or Fraunhofer features of the solar spectrum. That allows them to take recent measurements of the Ca^+ index as a measure of total ultraviolet irradiance. To estimate ultraviolet emission during the Maunder minimum, it is then simply necessary to use the measured Ca^+ emission from stars like the Sun, but showing no magnetic activity, to represent what the real Sun must have been doing in the seventeenth century.

The outcome is not on the face of things sensational. The total irradiance of the Sun during the Maunder minimum would have been 0.2 per cent less than that of the present quiet Sun, but the decrease of ultraviolet flux would have been much greater. Thus Lyman- α radiation would have been reduced by no less than 64 per cent (compared with the quiet Sun) during the Maunder minimum. Total ultraviolet output would have been more than 1 per cent less.

So does the altered output of energy from the Sun account for the severe winters of the Maunder minimum? Lean *et al.* remark that a global model of the climate predicts a temperature decline of 0.46 °C for a decrease of solar irradiance by 0.25 per cent, which is in itself a large fraction of the estimated average cooling (between 0.5 and 1.0 °C) during the Little Ice Age. They would have been even nearer the mark if they had followed the notion that the diameter of the Sun decreases during quiet spells (which should be measurable at the epoch free of sunspots).

The prolonged absence of sunspots would nevertheless have affected the ozone column in the atmosphere. On the basis of what they have learned about the reduction of ultraviolet flux, Lean *et al.* say that the total ozone column may have been reduced by between 4 and 8 per cent. With luck, that may account for the largest estimates of mean temperature reduction during the Little Ice Age. But nothing is as yet certain.

There remains the question of whether the next Maunder minimum will arrive in time to avoid a global carbon tax. Lean *et al.* have nothing to say on the subject, but there is a clue in the article by Baliunas and Jastrow, who say that roughly a third of a sub-sample of their Sun-like stars appeared to be magnetically quiet at any time. Allowing for the Maunder minimum and earlier and longer events centred on the fifteenth century, it seems that our own Sun has been spot-free for about a third of this millennium.

The statistics are not yet good enough to suggest how long climatic minima last, or how long are the intervals between them, but if the crunch-time for global warming comes in around 2050, the five solar cycles between now and then hardly seem enough to ensure safety. The moral: a better understanding of the Sun might now have practical value.

John Maddox

New recipe for a wee DRAM

Stephen K. Streiffer and A. I. Kingon

As electronics technology moves to ever-increasing densities and speeds, it is necessary to shrink the footprint of the integrated capacitors that represent digital bits in devices such as dynamic random access memories (DRAMs), and to integrate previously discrete components such as the decoupling capacitors used for noise suppression in high-frequency digital applications. Unless new architectures are developed, the charge stored per capacitor must also remain fairly constant from device generation to generation, so as to ensure reliable operation with a reasonable signal-to-noise ratio. Clearly these conflicting design rules imply that cell capacitance per unit area must increase, even as conventional integrated dielectrics based on silicon dioxide and Si_3N_4 approach their scaling limits. On page 215 of this issue, Cava *et al.*¹ describe the synthesis, through TiO_2 substitution into Ta_2O_5 , of an attractive replacement candidate.

Shrinking strategies

To increase the capacitance per unit of projected area (footprint) occupied by an integrated capacitor structure, three strategies may be pursued. First, one may simply decrease the thickness of the dielectric layer, as capacitance is inversely proportional to electrode separation for a planar device. But with the introduction of 64-Mbit DRAM designs, traditional dielectrics such as amorphous silicon oxide and silicon oxide-nitride-oxide heterostructures will reach practical limits, given by such factors as minimum thickness required to form a pinhole-free layer and to prevent significant leakage through electron tunnelling.

Second, one may increase the capacitor area per footprint area of the cell by introducing such three-dimensional structures as trenches, stacks, fins and crowns, or by introducing microstructural roughness by growing the dielectric on a rough bottom electrode². This approach is bounded from two directions — by the increase in leakage current with increasing roughness, and by the depth-of-field limitations of photolithography and other planarity requirements on device integration and circuit fabrication. This approach should provide sufficient stored charge per bit using currently available materials for the 256-Mbit and perhaps 1-Gbit generations.

The final strategy is clear: use of new materials with higher permittivities as the capacitor dielectric. But introduction of novel materials into a semiconductor fabrication facility is not approached lightly, given the sensitivity of semiconductor devices to contamination and the billion-

dollar investment that manufacturing facilities represent. Clever manipulation of device geometry has postponed this eventuality and greatly prolonged the service lifetime of the standard integrated dielectrics. Inevitably, though, the silicon-based materials currently in use will have to be replaced by advanced materials with much higher dielectric constants.

The foremost long-term candidates for this application are all members of the class of perovskite-derived ferroelectrics, including SrTiO_3 , BaTiO_3 and other compounds, with relative permittivities (or dielectric constants, ϵ_r) ranging from about 200 to well into the thousands (SiO_2 has $\epsilon_r=4$; Si_3N_4 has $\epsilon_r=7$). $(\text{Ba,Sr})\text{TiO}_3$ ($\epsilon_r=300$ –800) is perhaps the leading contender in this class, and most of the main DRAM manufacturers in the United States and Asia are devoting much research to it. For Ba/Sr ratios chosen so that the Curie point is pushed below operating temperatures, it benefits from low frequency dependence and excellent temperature stability of its permittivity.

Still, substantial barriers stand in the way — poor understanding of material properties and their variability (particularly leakage current and time-dependent polarization phenomena, which affect the voltage retention and switching speed of the capacitor); the lack of a well-established electrode technology, and process integration with the underlying silicon integrated-circuit technology; and questions about the long-term reliability. Film fabrication by chemical vapour deposition is the accepted way to achieve sufficient step coverage at the length scales and aspect ratios that will be dictated by future DRAM design rules. The process has been demonstrated for the ferroelectrics^{3–5}, but scale-up to industrial size for these oxides is only now beginning and presents its own challenges. Last and by no means least, these materials would require the introduction of barium and strontium into the fabrication process — relatively unfamiliar elements with unknown consequences.

Single-component oxides are the primary short-term competitors of the ferroelectrics, generally offering far fewer process integration problems, but with much lower relative permittivities (in the range of 20 to 100). Of these, Ta_2O_5 has been mooted for many years^{6–8}. Tantalum is already accepted in manufacturing facilities, and chemical vapour deposition of its oxide is well established. However, its relative permittivity is at best only 35, and when integrated directly onto a polysilicon bottom electrode, an SiO_2 interfacial layer is formed that limits the maximum attain-

able capacitance of the heterostructure. This offers just one device generation beyond current dielectrics, not enough to warrant extensive commercialization.

Cava and co-workers¹ may now have shifted this materials equation. By incorporating TiO_2 (another acceptable manufacturing material) into Ta_2O_5 , they have succeeded in substantially raising the permittivity of the compound, peaking with $\epsilon_r=126$ at 1 MHz for substitution of 8 per cent TiO_2 . If the permittivity increase can be replicated in thin films with a suitable bottom electrode, and other materials properties such as leakage current are acceptable, it would make possible a reduction in capacitor area satisfactory for at least two future DRAM generations, using a dielectric that may be more readily accommodated into existing strategies for device fabrication.

Structural change

How does it work? That question cannot yet be answered properly. The dielectric constant of polycrystalline TiO_2 reaches about 100, so it is attractive as a dopant simply on the basis of its own polarizability (pure TiO_2 itself is not of use because it reduces easily, leading to unacceptably large leakage currents). The authors note that the observed permittivity increase occurs with a change in crystal structure of the oxide to a monoclinic modification and only with TiO_2 substitution. So they propose that a structural distortion specific to Ti incorporation into the Ta–O framework leads to the increase in dielectric constant. The crystal chemistry of Ta_2O_5 is complex, however, and the specifics of the phenomenon warrant more investigation.

Ultimately, the choice of dielectric for commercialization will depend on dielectric constant and other properties related to capacitor performance, and on ease of incorporation into a mass-produced device. It remains to be seen whether thin-film Ta_2O_5 – TiO_2 will present enough of an advantage over current materials, and have a sufficient manufacturability edge over ferroelectrics, to make the transition to market. □

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Synapses take the rap

Stanley C. Froehner and Justin R. Fallon

ANY savvy investor knows that what really counts is not what you make but where you put it. In the realm of cell–cell communication, synapses are the premier example of deploying resources for maximal return. The gold standard of synapses, the neuromuscular junction, covers only about 1/10,000 of the muscle cell surface, but all of the molecular components necessary for synaptic transmission, including the postsynaptic nicotinic acetylcholine receptor (AChR), are clustered at this site¹. A central issue in neurobiology is understanding the mechanisms responsible for assembling these remarkable specializations. Now, results from a collaboration between the laboratories of Josh Sanes and the late John Merlie (page 232 of this issue²) show that rapsyn, a protein closely associated with nicotinic acetylcholine receptors, is not only involved in receptor clustering but may also be important for molecular specializations on both sides of the synapse.

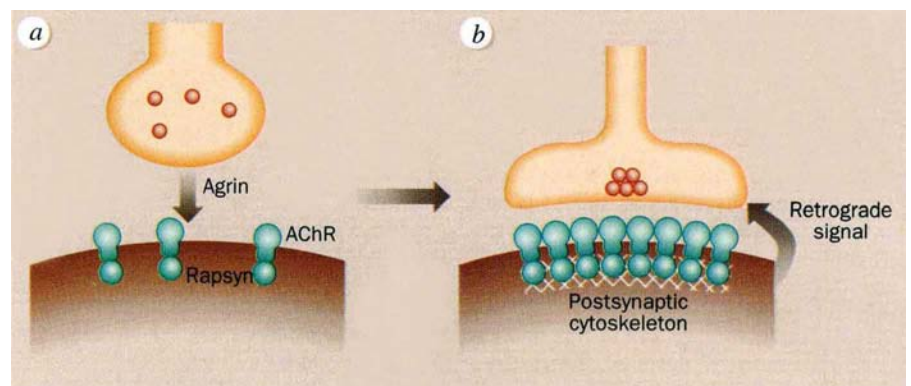
The differentiation and alignment of the pre- and postsynaptic machinery is a cooperative process requiring the continuous exchange of information between the two cells (see figure). Signals from the nerve induce the formation of the postsynaptic apparatus. Two such nerve-derived signals are responsible for the localized accumulation of AChRs. Acetylcholine-receptor-inducing activity (ARIA) promotes synthesis of AChRs³ and may mediate the selective activation of receptor genes in synaptic nuclei⁴. Independently, a protein called agrin initiates AChR aggregation by binding to its own receptor and activating an intracellular cascade culminating in the clustering of AChRs as well as several other synaptic molecules⁵. The mechanisms mediating presynaptic differentiation are poorly understood, but undoubtedly involve information feedback from the postsynaptic cell.

For some time, it has been thought that AChRs are anchored at the synapse by interaction with cytoskeletal and peripheral membrane proteins, and that agrin induces clustering at least in part by promoting the assembly of such postsynaptic cytoskeletons. Rapsyn (receptor-associated protein at the synapse, also known as the 43K protein) is a major component of the submembrane complex⁶. First identified in *Torpedo* postsynaptic membranes, rapsyn is a peripheral membrane protein closely associated with the nicotinic receptor. Evidence that it participates in clustering comes from coexpression studies in heterologous cells^{7,8}. AChRs expressed alone are diffusely distributed over the cell surface, but become organized into

discrete clusters when coexpressed with rapsyn; indeed, rapsyn has inherent clustering activity, and can form membrane-associated clusters even in the absence of the AChRs. But although rapsyn has the expected profile of an anchoring protein, its function at the synapse, and whether or not it is part of the agrin pathway, was unknown.

Gautam *et al.*² have tackled these problems using mice in which rapsyn expression was eliminated by targeted disruption of its gene. First, the obvious question — are postsynaptic specializations affected in

al folds along with rapsyn and the AChR; it is also associated with dystroglycan, a receptor for agrin¹⁰, and the syntrophins, one isoform of which is synapse-specific¹¹. The synaptic concentration of each of these components is dramatically reduced or even eliminated in the mutant. So rapsyn must interact, directly or indirectly, with components of the utrophin complex. These *in vivo* observations agree with results of coexpression studies showing that dystroglycan targets to recombinant rapsyn-induced clusters¹², although the biochemical interactions required for targeting are not yet known. Finally, effects of the rapsyn mutation extend to basal lamina, where the synaptic form of acetylcholine esterase is greatly reduced. In all, then, organization of much of the post-



Steps in synaptic differentiation. *a*, Agrin secreted by motor neurons induces clustering of nicotinic acetylcholine receptors (AChRs) and organization of the utrophin/dystrophin cytoskeleton in a rapsyn-dependent mechanism. *b*, The organized postsynaptic apparatus in turn generates signals that promote presynaptic differentiation. The components of this hypothetical retrograde pathway are unknown, but at least some of them are also rapsyn-dependent.

these mutants? The answer is clearly yes. Although homozygous mutant mice showed no gross abnormalities, they had impaired movement and died within hours of birth. Upon examination of neuromuscular junctions, the reason for these defects became clear — the AChR clustering so readily apparent at normal nerve–muscle contacts was absent in the mutant. Second, rapsyn is required for agrin-induced AChR clustering on cultured muscle cells, and thus must lie directly in the signalling pathway activated by agrin. In contrast, the selective transcription of AChR genes at synaptic nuclei is unaffected in the mutant. So the agrin/rapsyn clustering pathway must be distinct from the signalling events that regulate AChR expression.

These results are clear evidence that rapsyn is essential for AChR clustering at synapses, but the mutants also reveal that rapsyn is much more widely involved in structuring postsynaptic specializations. Among the proteins concentrated in the postsynaptic membrane are dystrophin and utrophin⁹. Utrophin may be especially important for AChR clustering, as it is restricted to the crests of the postjunction-

synaptic apparatus depends on the proper expression of rapsyn.

A completely unexpected consequence of the rapsyn mutation was aberrant presynaptic differentiation. Normal and mutant terminals were quite similar, exhibiting close apposition of pre- and postsynaptic membranes, an intervening basal lamina and typical patterns of synaptic vesicles. But nerve endings in mutant mice formed long branches that lacked the distinct arbours characteristic

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of normal terminals, suggesting defects in expression or organization of muscle-cell adhesion or signalling molecules. Rapsyn thus emerges in a new role and in largely uncharted territory — the mechanisms guiding the precise alignment of pre- and postsynaptic membranes. It has long been speculated that nerve-induced postsynaptic specializations in turn generate retrograde signals required for furthering presynaptic differentiation (see figure). The placement of rapsyn in this chain of events was unexpected but opens the way for a molecular attack on this problem.

What next? One outstanding question is the identity of rapsyn's cytoskeletal/signalling partners. Although rapsyn is characterized by a zinc finger and a leucine zipper, which are probably sites for protein-protein interactions, it shares

no convincing homology with any known proteins. So further examination of the synaptic actions of rapsyn, including the identification of proteins associated with it, might provide information on signalling pathways that regulate both postsynaptic and presynaptic events. Given the diverse consequences of the rapsyn mutation, we can look forward to the unveiling of other classes of signalling molecules that also rely on being in the right place to give maximal return. □

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NITRIC OXIDE

No endothelial NO

Solomon H. Snyder

THE more the biological functions of nitric oxide are investigated, the more of them emerge. The stream of new findings has continued unabated for some eight years, sometimes delivering surprises with clinical implications. The latest turn of events appears on page 239 of this issue¹, where Huang *et al.* describe the consequences of knocking out the mouse gene encoding nitric oxide synthase in endothelial cells — nitric oxide produced by such cells is a potent vasodilator, thought to be involved in the regulation of blood pressure and local blood flow, and abnormalities in its production occur in atherosclerosis, diabetes and hypertension.

First, some background. The three principal forms of nitric oxide synthase (NOS) are the neuronal (nNOS or NOS-1), the macrophage or inducible (iNOS or NOS-

2) and the endothelial (eNOS or NOS-3). The names can be misleading (see table). For example, iNOS not only occurs in macrophages but in several cell types including hepatocytes, chondrocytes, endothelial cells and fibroblasts. eNOS is not restricted to the endothelium of blood vessels but exists in the epithelium of several tissues, including the bronchial tree; it has also been localized to neurons in the brain, especially the pyramidal cells of the hippocampus, where it may function in long-term potentiation, a model of learning and memory². nNOS also occurs in skeletal muscle³, where Bredt and associates⁴ have found it complexed with dystrophin, and to be absent in Duchenne's muscular dystrophy, which perhaps accounts for symptoms of the disease.

The biological functions of nitric oxide

have been clarified considerably by the use of mice with various forms of NOS inactivated through gene knockouts created by homologous recombination. Considering nitric oxide's ubiquitous roles, the viability of such knockouts was questionable: not least because nNOS occurs in the nerves that mediate penile erection, and NOS inhibitors block erection⁵, it was thought that null mutant nNOS (nNOS⁻) mice would not procreate. As it turned out, however, nNOS⁻ animals do breed and appear to be generally normal⁶. But they do have dilated stomachs with a constricted pyloric sphincter, and so provide a valuable model for the clinical disease infantile hypertrophic pyloric stenosis⁶. nNOS⁻ mice are resistant to brain damage caused by vascular strokes, confirming that nitric oxide is crucial in mediating stroke damage⁷. And, earlier this year, studies of nNOS⁻ mice established the importance of this form of the enzyme in behaviour⁸ — nNOS⁻ males are highly aggressive towards other males, to the extent that they will kill their wild-type littermates if left unattended, and they display similarly striking inappropriate and excessive sexual behaviour.

iNOS⁻ mice have clarified the participation of nitric oxide in inflammatory responses^{9,10}. Nitric oxide made by macrophages had long been implicated in the tumoricidal and bactericidal actions of these cells, but studies with NOS inhibitors had been inconclusive. iNOS⁻ mice have markedly reduced defences against microorganisms such as *Listeria*⁹ and *Leishmania*¹⁰, and against the proliferation of lymphoma tumour cells. On the other hand, these animals are resistant to carrageenan inflammation¹⁰ and hypotension elicited by endotoxin^{9,10}.

Now, Huang *et al.*¹ report on eNOS⁻ mice. That eNOS is involved in regulating blood pressure had long been implied by the ability of NOS inhibitors to raise blood pressure. But most NOS inhibitors are nonspecific. Conceivably, their inhibition of nNOS, not eNOS, raises blood pressure, because nNOS occurs in neurons in the adventitial layer of vessels. Many different mediators have effects on the cardiovascular system, so one would not be surprised if knocking out eNOS had no effect on blood pressure.

The eNOS⁻ mice of Huang *et al.* are viable, reproduce normally and are largely indistinguishable from wild-type animals in general appearance and behaviour. The classic role of nitric oxide as 'endothelium-derived relaxing factor' is substantiated by these mice, whose aortic rings display no relaxation in response to acetylcholine and are unaffected by treatment with the NOS inhibitor L-nitroarginine. Their vascular smooth muscle is normal (norepinephrine constriction of blood vessels and its reversal by sodium nitroprus-

Phenotypes of mice deficient in nitric oxide synthase (NOS)

NOS subtype	Phenotype
Neuronal (nNOS, type 1)	Pyloric stenosis ⁶ Resistant to vascular stroke ⁷ Inappropriate, excessive sexual and aggressive behaviour ⁸
Inducible (iNOS, type 2)	Normal hippocampal long-term potentiation ² and cerebellar long-term depression ¹⁴ More susceptible to <i>Listeria</i> ⁹ and <i>Leishmania</i> ¹⁰ infection and lymphoma cell proliferation ⁹ Resistant to endotoxin hypotension ^{9,10} and carrageenan inflammation ¹⁰
Endothelial (eNOS, type 3)	Deficient acetylcholine vasodilation ¹ Elevated mean blood pressure ¹ L-nitroarginine-induced hypotension ¹

side is the same in eNOS⁻ and wild-type animals). Mean blood pressure is 35 per cent higher in eNOS⁻ than control animals, confirming that endothelial nitric oxide participates in normal blood-pressure regulation. Indeed, the pronounced hypertension of the eNOS⁻ animals implicates nitric oxide as the primary determinant of physiological blood pressure and establishes it for sure as the major physiological vasodilator.

Surprisingly, Huang *et al.* find that L-nitroarginine, which raises blood pressure in wild-type mice, dramatically lowers blood pressure in eNOS⁻ animals. This reflects NOS inhibition, as it is not evident with D-nitroarginine and is reversed by L-arginine treatment. Presumably some form of NOS other than eNOS normally contributes to increases in blood pressure. nNOS in adventitial neurons of blood vessels is the best candidate, although nNOS in skeletal muscle could also be participating. Nitric oxide may mediate the contraction of 'fast' muscle fibres¹, and massive skeletal muscle contraction increases blood pressure.

There remain unanswered questions. Numerous studies indicate that nitric oxide is not the only endothelial-derived relaxing factor, as in many vessels a substantial component of vessel relaxation is unaffected by NOS inhibitors^{11,12}. Carbon monoxide is one possibility — the carbon-monoxide synthesizing enzyme, haem oxygenase II, is localized to endothelium, and inhibitors of haem oxygenase reverse the portion of acetylcholine-induced vessel relaxation that is resistant to nitroarginine¹³. Numerous localizations of haem oxygenase II to autonomic nerves parallel those of nNOS, suggesting that nitric oxide and carbon monoxide may act in concert throughout the body. Mice with knockouts of genes for the respective biosynthetic enzymes should clarify the possibly synergistic functions of these two mediator molecules. □

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High-speed diamond

Robert W. Cahn

THERE are two quite distinct routes to making synthetic diamond: the high-pressure catalytic approach perfected at General Electric in the United States and de Beers in South Africa in 1955–60, and the chemical vapour deposition (CVD) method, first patented in the United States in 1958 and developed seriously some years later. The high-pressure route is mostly used to manufacture large quantities of fine diamond grit for use in grinding, whereas the CVD method of making thin sheet and coatings, using (nowadays) a plasma excited by microwaves and a dilute hydrocarbon charge in hydrogen

then slow migration of any one growth interface becomes less of a handicap. They point out that the deposition of CVD diamond is always heterogeneously nucleated, for instance at scratches on a tungsten substrate: a coherent 'string' of diamond crystallites is then deposited along such a scratch and thereafter these can grow sideways, giving (in the situation shown in Fig. 1) a sixfold enhancement in the effective growth rate. If deposition were homogeneous, the whole surface would be covered simultaneously and there would be no enhancement of the intrinsic growth rate. The team then went on to apply this recognition to making deposits on wires and fibres. A fine fibre has a high specific surface area, and diamond deposited on it initially grows fast. Figure 2 shows CVD diamond grown on a crossed grid of tungsten wires of 100 µm diameter.

The key insight of the paper comes next, and is clarified by Figs 1 and 3. If the period of such a grid of wires or fibres is made small enough, a configuration such as that in Fig. 2 can 'fill in' very quickly and generate a continuous sheet, albeit not a very smooth one.

The next step was to make a small-diameter (about 150 µm) spiral of tungsten wire with a period of about 70 µm, by winding on a ceramic fibre (they could have broken open an electric light bulb and used the filament), and to deposit diamond on that to make a tube, as sketched in Fig. 3. Effectively, the growth rate is multiplied by the number of turns. The authors go on to make a detailed calculation of the effective deposition rate for a three-dimensional variant of the configuration shown in Fig. 2, made up of 200,000 50-mm lengths of 10 µm wires; a plasma can readily be made large enough to coat the whole of this array simultaneously, and for a 1 µm per hour intrinsic growth rate, a deposit weighing 330 g in all can be deposited in 50 hours. So the rate is 6.6 g per hour, which compares with 0.11 g h⁻¹

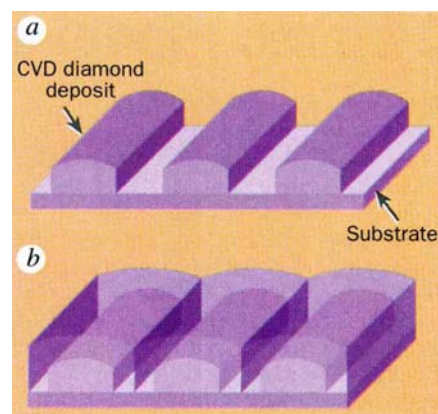


FIG. 1 Schematic diagram of heterogeneous diamond nucleation at scratches on a substrate and subsequent growth. *a*, Initial stage; *b*, continuous diamond coating. (From ref. 1, with permission.)

carrier gas, is now beginning to find uses in producing cutting tools, wear-resistant surfaces, heat sinks and loudspeaker diaphragms. Synthetic gems are notable by their absence; they can be made by the high-pressure approach, but do not make economic sense. CVD diamond production likewise suffers from an economic penalty, that of exceedingly slow growth rates. These are typically one micrometre per hour if the diamond deposit is to be of good quality — and that is at the upper end of the practical temperature range for deposition, which is 300–1,000 °C. A new study published by Partridge and co-workers in the *Journal of Materials Science*¹, simple in conception but ingenious in execution, now offers the prospect of a manifold increase in the effective growth rate.

Partridge's team started from the recognition that if multiple growth interfaces can be brought into play,

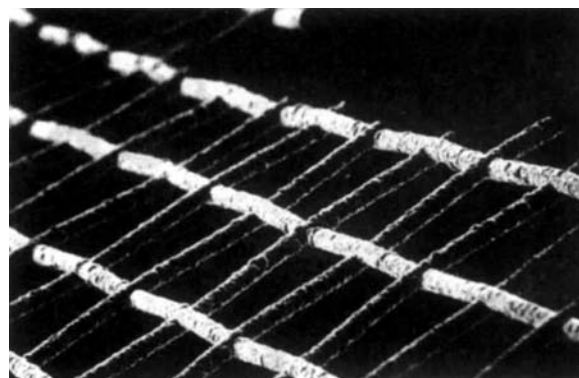


FIG. 2 Scanning electron micrograph of diamond-coated tungsten wire grid. (From ref. 1, with permission.)

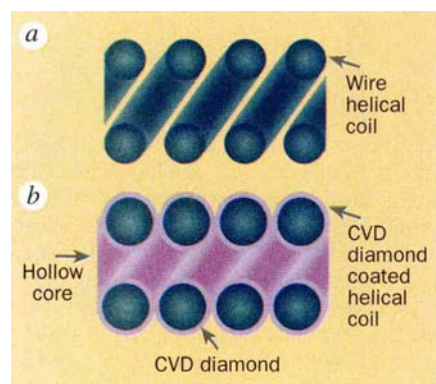


FIG. 3 Schematic diagram of diamond deposition on to a helical coil (a), showing geometry after diamond coating has just begun to join up (b). (From ref. 1, with permission.)

on a plane wafer 20 cm across. With a fine helical coil the same size as the wire assembly, the effective growth rate could be as much as 13 g h^{-1} before the turns join up.

It is clear from the paper that the underlying objective was to find an affordable way of exploiting the remarkable mechanical properties of diamond in the reinforcing constituent of a fibre-composite. The kind of configurations shown in Figs 2 and 3 could serve for this purpose. A calculation at the end of the paper shows that diamond reinforcement could be made by the microwave or hot-wire variant of CVD for a sum in the range \$4–15 per gram compared with a present cost of \$2.7–5.3 per gram for the high-quality silicon carbide fibre which is the current aristocrat in the composites field. Diamond would appear to have become a realistic reinforcing fibre for high-tech uses.

The paper by Partridge's group meshes nicely with two other papers, by Ting², in the same issue of the *Journal of Materials Science*. He shows the effectiveness of ultrafine carbon fibres, only $0.2 \mu\text{m}$ in diameter, as substrates for diamond growth (hitherto, silicon or metals have been preferred substrate materials. It is necessary either to etch the substrate with atomic hydrogen or, even more effectively, to abrade the substrate with fine diamond dust which then remains to provide favourable nuclei. With such fibres, bursts of explosive diamond growth are encountered in a microwave apparatus; the microwaves generate the requisite energy. Carbon-carbon composites, consisting of

a fibre array filled with graphitic carbon, also form an effective substrate. Presumably — although the author does not actually say so — the objective is to generate a diamond-loaded, high-strength composite.

There is now clear evidence that CVD diamond, together with so-called diamond-like carbon (which is a poor man's substitute), is approaching large-scale application. The science of the CVD process itself was treated in a Royal Society Discussion two years ago³; the twists and turns of the commercial development of the CVD approach, and the progressive discovery of realistic applications, are concisely surveyed by Fujimori⁴. Good wear resistance is another property that is likely to be exploited with CVD diamond

surface coatings⁵. Fujimori himself⁶ developed large diamond-coated alumina diaphragms for loudspeakers, exploiting the very high speed of sound in diamond, thereby raising the upper frequency cut-off from 35 to 50 kHz. Later, as described in his book chapter, he was able to make free-standing diamond diaphragms by deposition on silicon, followed by etching away of the substrate, and raised the cut-off to 80 kHz. A loudspeaker with a diamond diaphragm must be an ad-writer's dream product. □

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GEOPHYSICS

Dynamo model at a turning point

George Backus

In 1600, W. Gilbert¹ absolved the pole star Polaris of responsibility for the Earth's magnetic field, **B**. He found that a uniformly magnetized sphere produced the same latitude dependence of the angle between the field and the local vertical as was being reported by the navigators of the time. Thereafter scientists have sought the origin of the magnetic field inside the Earth. On page 203 of this issue, Glatzmaier and Roberts² make a considerable contribution to that quest: they have captured a field reversal in their numerical fluid dynamo.

Almost a century after Gilbert's discovery, Halley³ rejected magnetized surface rock as the source of **B** by noting that in Europe the pattern of deviations from geographical north appeared to be drifting westwards at a rate which would circle the Earth in about a millennium. Halley hypothesized as the seat of the field a sphere inside the Earth, capable of moving relative to the surface. In the early twentieth century Einstein called the origin of **B** one of the fundamental unsolved problems in physics.

Further progress was sluggish until, in 1919, Larmor⁴ suggested that a fluid dynamo might generate the magnetic fields recently discovered in sunspots, and geophysicists carried the idea inside the Earth. The molten iron in the Earth's outer core will not support permanent magnetization, but it is a good electrical conductor. Fluid flowing across magnetic field lines induces electric currents. These will exert a Lorentz force on the fluid and also generate a magnetic field. Could the Lorentz force and the buoyant force of convection together move the fluid so that the induced electric currents generated the original magnetic field? Fluid dynamos would not be perpetual motion

machines because convective buoyancy would supply the energy lost to electrical resistance. Such a system would be a 'full' fluid dynamo.

Disappointingly, in 1934 Cowling⁵ showed that if **B** was steady and axisymmetric, even a 'kinematic' dynamo seemed to be impossible. That is, the field could not be maintained against ohmic decay by any fluid velocity field whatever, much less one generated by a realistic buoyancy mechanism. In the 1940s, Blackett⁶ responded by proposing an extra term in Maxwell's equations to describe generation of **B** by rotating masses (an approach that turned out not to be fruitful), and Elsasser⁷ revived the search for kinematic dynamos by suggesting that non-axisymmetric forms of **B** be tried. Neither the mathematical nor the computer technology of the day was up to the task, and it was another 12 years before mathematically rigorous (and quite non-physical) examples of kinematic fluid dynamos were constructed. By 1973, Gubbins⁸ had produced convincing numerical examples of kinematic dynamos with physically reasonable velocity fields.

Meanwhile Parker⁹, pursuing Elsasser's suggestion, began the study of what are now called 'mean field dynamos', two-scale dynamos invoking the hypothesis that an essential part of the generation of the magnetic field is by very small-scale fluid motions which can be parametrized just as viscosity parametrizes the fluid friction produced by molecular collisions. The two-scale hypothesis simplified the kinematic and full dynamo equations enough that both could be solved. However, no one could verify the hypothesis experimentally, and the mathematical arguments were suggestive but inconclusive. Experimental fluid dynamos are still

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out of reach, given the available funding. Planetary dimensions are not required, but numerical and theoretical studies of kinematic dynamos suggest that in liquid sodium (a possible medium for models), small-scale eddies would not regenerate **B** unless the product of their linear size and helical speed were larger than about $20 \text{ m}^2 \text{ s}^{-1}$.

The experimental difficulties and the doubtful theoretical foundation of mean-field dynamos explain the importance of Glatzmaier and Roberts' work². Their computer code for the full dynamo problem carries two representations of the magnetic, velocity and temperature fields, one pointwise and the other spectral (a truncated spherical harmonic expansion). The nonlinear terms are computed from the pointwise representations, and the time steps from the spectral representations. Omission of inertial terms in the momentum equation suppresses Alfvén waves and permits larger time steps. Several dimensionless parameters enter the full dynamo problem, and the part of parameter space where their code has adequate stability and resolution excludes the Earth. However, the authors suggest that they may have reached the asymptotic range where further parameter changes have only minor effects. The original dynamo used by Glatzmaier and Roberts omitted a solid inner core, and was found to be chaotic. Numerical work by Hollerbach and Jones¹⁰ on a simplified axisymmetric mean-field dynamo suggested that a solid inner core would stabilize the dynamo, and Glatzmaier and Roberts found this to be so.

The new dynamo simulation differs in several ways from the geomagnetic observations. The field reversal is faster, and its dipole/non-dipole ratio is weaker than in the Earth. This model dynamo produced one reversal during its 40,000 simulated years of running time, whereas the record of rock magnetism indicates that the time between reversals has been as long as 35 million years (the Cretaceous quiet zone) and is typically a few hundred thousand years. However, palaeomagnetic data do show some shorter intervals between reversals.

The work of Glatzmaier and Roberts is a promising start in a field where much

remains to be done. They can now test the mean field hypothesis numerically. Speeding up the code may permit investigations on geological timescales (telling us, for instance, whether the Cretaceous quiet zone diagnoses lower-mantle tectonics) and on the human scale needed to study angular momentum transfer between crust and mantle or to seek numerical evi-

dence for the magnetic jerks of Ducruix *et al.*¹¹. They will not decide whether fluid dynamos can run forever, but then neither has the Earth. □

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ECOLOGY

New cog in the nitrogen cycle

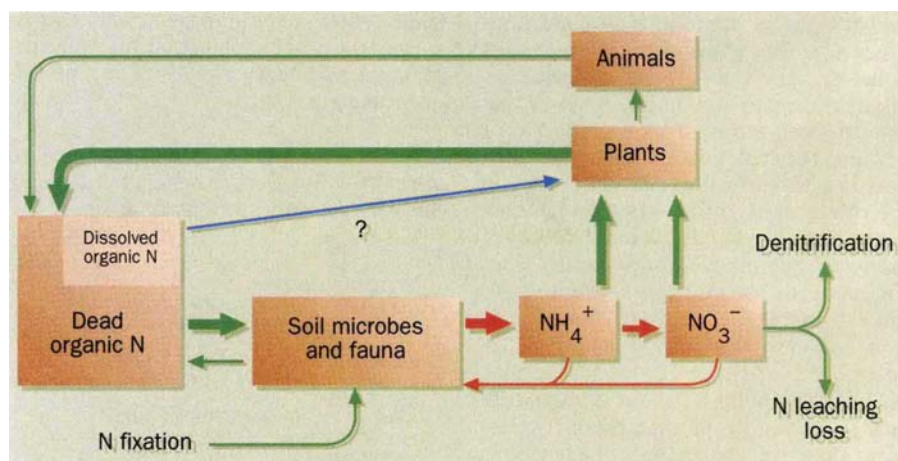
F. Stuart Chapin III

THE paper by Northup *et al.*¹ on page 227 of this issue shows for the first time that a pine (*Pinus muricata*) can strongly influence release of dissolved organic nitrogen (DON) in soils through the production of polyphenols in leaf litter. This result provides fresh evidence that DON has a pivotal role in terrestrial nitrogen cycling and suggests a new explanation as to why plants on infertile soils often have high concentrations of phenols.

Because the availability of nitrogen limits the productivity of most agricultural² and natural³ terrestrial ecosystems, there has been extensive research on the controls over its supply, leading to the generalization that the mineralization of organic nitrogen to ammonium and its subsequent oxidation to nitrate are the major bottlenecks restricting supply of the nutrient to plants (see figure). In relatively fertile ecosystems, where most research has been conducted, there is abundant support for this generalization. In infertile ecosystems, however, measured rates of net microbial production of inorganic nitrogen are often less than half the observed rates of nitrogen acquisition by plants, suggesting that plants tap some pool of organic nitrogen as well as the

inorganic nitrogen released by mineralization¹. Alternatively, plant roots or their mycorrhizal associates may compete with soil microbes and absorb more inorganic nitrogen than indicated by standard measurements of net microbial production of inorganic nitrogen, which are usually conducted in the absence of plant roots. These measurements may, therefore, consistently underestimate the availability of inorganic nitrogen to plants in the field.

Northup *et al.*¹ contribute to the developing body of evidence that organic nitrogen is a major and direct source for plants in nitrogen-limited ecosystems, and that the conventional view of nitrogen cycling in these ecosystems is incomplete (see figure). There is increasing evidence that much of the nitrogen absorbed by plants from infertile and/or highly organic soils is organic in origin, short-circuiting the mineralization step which has traditionally been considered to be the limiting factor in terrestrial nitrogen cycling. Arctic plants readily absorb amino acids⁴ and grow more rapidly on organic than on inorganic sources of nitrogen⁵. In addition, ectomycorrhizal and ericoid mycorrhizal fungi, which are common in infertile soils, directly break down pro-



The main fluxes (arrows) and pools (boxes) of nitrogen in terrestrial ecosystems. Arrow thickness is proportional to the magnitude of net flux, and red arrows indicate microbial controls that are conventionally believed to limit overall cycling rates. The path from the pool of dead organic nitrogen to plants short-circuits these conventional microbial controls, and could be an important route of nitrogen transfer in infertile ecosystems.

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Vegetation in the pygmy forest community studied by Northup *et al.*¹. The small but old trees in the foreground exist on highly acidic and infertile soil, and contrast with the much larger specimens of the same species and age rooted in more fertile soil only a short way away. The authors suggest that the stunted community's survival is linked to their greater production of polyphenols, which enables them to use greater amounts of dissolved organic nitrogen as a nutrient source.

teins in soil organic matter, absorbing and transferring the resulting amino acids to their host plants without nitrogen being mineralized to inorganic form⁶.

Northup *et al.* now show that pine trees growing on infertile soils produce litter with high rates of production of DON (relative to inorganic nitrogen), that could then diffuse to mycorrhizal hyphae or plant roots, thus increasing the supply of DON to plants. They suggest that polyphenols such as tannins mediate this enhanced DON mobilization — plants that can use phenol-bound organic nitrogen could enhance the supply of this form of the nutrient, providing them with a competitive edge over plants that preferentially use ammonium or nitrate as a nitrogen source.

The conventional explanation for high tissue concentrations of phenols is that these compounds defend plants against pathogens⁷ and herbivores⁸. Polyphenols are thought to occur in high concentrations in plants growing on infertile soils either because slow growth in these sites allows carbon to accumulate to levels that can support rapid phenol synthesis, or because the high cost of replacing nutrients lost to herbivores and pathogens in infertile ecosystems selects for high concentrations of phenols as plant defences. However, the plant-defence explanations for patterns of phenol concentrations among plant species have been questioned on two grounds — many herbivores are sensitive only to specific phenolic compounds⁹, and it is often difficult to demonstrate that production of plant defences constitutes a high cost in terms of reduced plant growth¹⁰.

Convincing tests of Northup and colleagues' hypothesis will require examination of at least two assumptions. The first is that the individual plant that produces

these compounds acquires more DON than competing plants, and that the benefit of increased DON absorption exceeds the cost of polyphenol production. It still remains to be shown that *Pinus muricata* is capable of absorbing organic nitrogen. The second assumption is that selection for phenols to enhance DON acquisition is independent of other factors, such as herbivory, that might have selected for high phenol contents in species or populations occurring on infertile soils. A plausible alternative to the hypothesis of Northup *et al.* is that plants have adapted to use organic nitrogen in an environment where other factors have selected for high phenol content and resulting release of phenol-protein complexes.

Even as it stands, however, Northup and colleagues' paper makes a substantial contribution to ecology. It offers a hitherto unregarded mechanism by which plants can short-circuit the mineralization step in the nitrogen cycle, and it suggests a novel explanation for patterns of plant secondary chemistry in natural environments. □

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DAEDALUS

Slick thinking

TANKER owners heroically claim that many oceanic oil slicks are not their fault at all, but are 'natural seepage' from submerged oil-bearing strata. They may have a point. Daedalus now proposes a new source of natural oil slicks.

His reasoning starts from the exhaust catalysts on modern cars, which convert hydrocarbons and oxygen into carbon dioxide and water. Some of them can work this trick quite fast, even at modest temperatures. Now a catalyst is always reversible; it speeds up a reaction just as much backwards as forwards. So, says Daedalus, under the right conditions an exhaust catalyst could take carbon dioxide and water, and turn them back into hydrocarbon and oxygen. Such an 'uphill' reaction would soon grind to a halt unless the hydrocarbon were removed as fast as it formed. One way to do this would be to run the reaction under water. The hydrocarbon, immiscible with water, would continuously rise away from the catalyst surface and float to the top, leaving the catalyst free to make more. The carbon dioxide needed could simply be dissolved in the water. The reaction, essentially combustion in reverse, would absorb heat strongly, and would probably go very slowly. DREADCO's chemists are now trying it, by submerging chunks of various exhaust catalysts in tanks of soda water. A slick of crude oil should slowly form on the water surface — a triumph of theory, perhaps, but hardly the answer to an oil-driller's prayers.

Daedalus, however, claims that nature has got there first. He points out the mysterious shortfall in the atmospheric carbon budget. Despite the millions of tons of carbonaceous fuel and thousands of square miles of tropical forest we are burning every year, the air and the ocean are not accumulating carbon dioxide as they should. Where is it going? Somewhere in the oceans, says Daedalus, a natural catalyst must be turning it back to oil, and launching it as oil slicks.

Whatever it is, the catalyst must be widely distributed. It may be a freshly precipitated mineral from the superheated 'black smoker' mid-ocean springs. It may be the shell of some ingenious mollusc, designed to mop up excessive carbon dioxide and save itself from dissolution to bicarbonate. DREADCO oceanographers are now scouring mineral collections and aquaria in search of it. The idea is not to augment the world's oil supply — at least not yet. They want to replace the vastly expensive precious-metal catalysts of car exhausts by a cheap, natural, organic product.

David Jones

Atlantic lava lakes and hot vents

SIR — Submarine lava lakes were first observed on intermediate- to fast-spreading ridges on the Galapagos ridge¹ and on the East Pacific Rise². They are the result of rapid eruption of very hot and fluid lava. There is a good correlation between high effusion rate, hot fluid lava, lava lakes and high spreading rate. Typical lava lake structures, such as lava pillars and collapsed roofs, have not been reported from slow-spreading ridges, although localized sheet flows accumulating in topographic lows³ were described on the AMAR segment of the Mid-Atlantic Ridge at 36° 27' N. We report here the discovery of two extensive lava lakes on the Mid-Atlantic Ridge and their possible implication for the magmatic and hydrothermal circulation beneath slow-spreading ridges.

During the DIVA1 diving cruise (1994) with the submersible *Nautilie* on the Mid-Atlantic Ridge near the Azores hotspot, two lava lakes were discovered at the central topographic high of the Lucky Strike (37° 17.5' N) and Menez Gwen segments (37° 50' N) (see figure). At Lucky Strike,

the lava lake is 300 m in diameter and lies at the bottom of a 1-km-diameter caldera. Several generations of very fresh lava form superimposed roofs and pillars several metres high. At Menez Gwen the lava lake measures 0.4 × 1.4 km and is centred in a 6 × 2-km axial graben covered with very recent pillow and lobate flows. A 200-m-high volcano covered with extremely fresh pillows is at the northern end of the graben.

The discovery of lava lakes at the central topographic highs of the segments (see figure) is consistent with the idea that a thick crust and shallow brittle-ductile transition zone characterize the middles of segments, whereas a thin crust and deep brittle-ductile transition zone are present near segment discontinuities⁴. Lucky Strike, the largest (1 km²) hydrothermal vent field on the Mid-Atlantic Ridge, is a ring encircling the lava lake within the caldera. At Menez Gwen the hydrothermal field lies in the graben, near the top of the new volcano with the most recent lava, 1 km north of the lava lake. The association of a topographic high,

hydrothermal field and lava lake suggests that the rapid localized effusion rate needed to create a lava lake is favoured by close proximity to the top of a shallow axial magma chamber. Thus, we speculate that a shallow magma-lens reflector and seismic low-velocity zone may exist beneath these two sites at the along-axis highs.

Compared with lava lakes on fast-spreading ridges, those on slow-spreading ridges are smaller, presumably reflecting a more localized magma chamber. We propose that lava lakes mark on the sea floor the position of the hottest domain and the shallowest part of the magma chamber, suggesting that this heat source has the morphology of a blow torch centred on the topographic high of the segment.

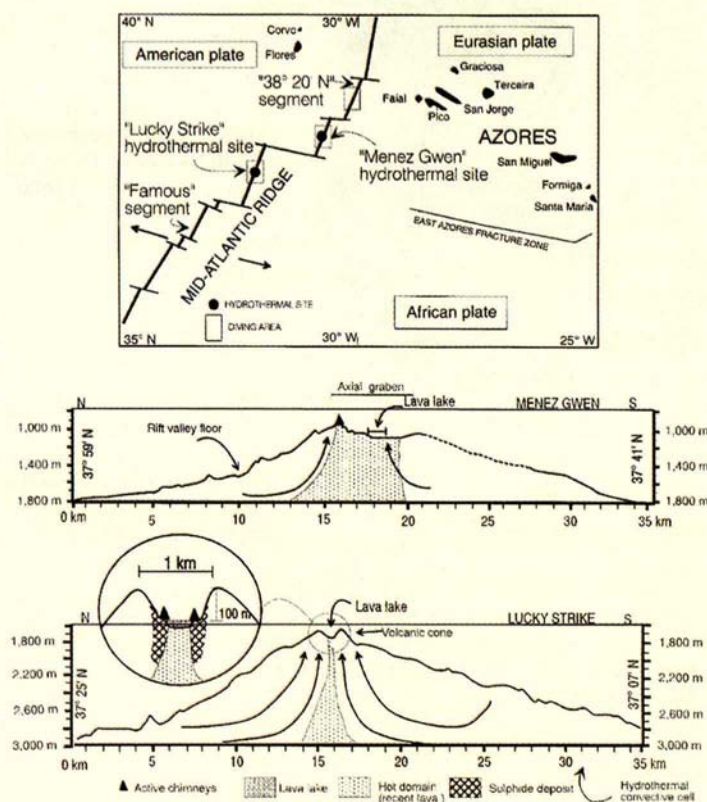
Elsewhere on the Mid-Atlantic Ridge, outside the area of influence of the Azores hotspot, the TAG and Snake Pit hydrothermal deposits (at 26° and 23° N, respectively) are not associated with lava lakes. They are, however, located at or near the topographic highs of ridge segments. Thus, as with Lucky Strike and Menez Gwen, their location is controlled by the hottest domain (with high magmatic budget) along the ridge.

The sulphide deposits around the lake can be considered as the trace of the upper part of the hydrothermal convective cells. At Lucky Strike their annular distribution suggests that major upwelling cells are radially distributed, and their exits are controlled by the high-permeability margins of the lava lake and the collapsed caldera. This suggests that, unlike the unstable two-dimensional convective systems on fast-spreading ridges, slow-spreading ridges with high magmatic budget (near a hotspot) are dominated by more stable three-dimensional convection centred on the volcanic topographic high. This is more compatible with the formation of very large sulphide deposits related to the succession of several hydrothermal episodes at the same location. This is also consistent with the model of two-dimensional passive upwelling under fast-spreading ridges and three-dimensional (diapiric) mantle upwelling under slow-spreading ridges⁵.

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Location of the two hydrothermal sites in the Azores domain of the Mid-Atlantic Ridge. Both sites are at the volcanic topographic high in the central part of the segment, where we have seen fresh lava flows and lava lakes. The Lucky Strike hydrothermal field encircles the lava lake, whereas the Menez Gwen field is apparently controlled by the most recent central volcano, about 1 km north of the lava lake. Fresh lava at the topographic high indicates a high magmatic budget, which in turn is compatible with a hot central domain (possibly a shallow magma chamber) controlling focused three-dimensional hydrothermal convective cells. Vertical exaggeration, 5.

Disentangling giant sperm

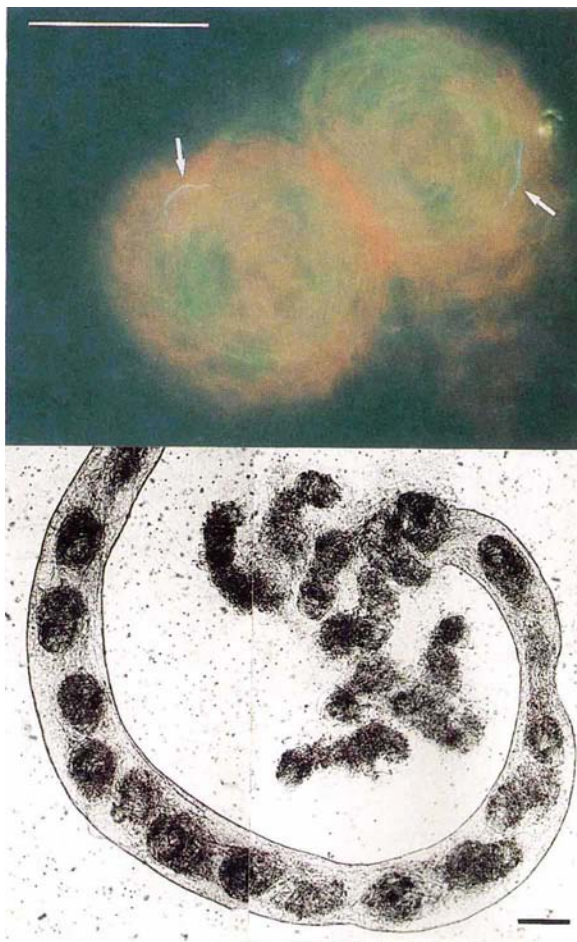
SIR — The theory of the evolution of sex suggests that the greater the investment in individual gametes, the more parsimonious males should be in transferring sperm to females. It has been argued that physiological constraints may limit the range of allocation strategies available to males; therefore, the evolution of giant sperm up to 2 cm long in *Drosophila hydei* and 6 cm in *D. bifurca*, 7 and 20 times as long as the male body, respectively, could appear as one of the great paradoxes of sexual selection. The selective value of these giant sperm has become a matter for controversial and growing debate^{1,2}.

Concerning the anisogamous pattern, *Drosophila bifurca* is the most extreme and impressive example known of how far evolution can shift in unexpected directions. During the 17 days needed by *D. bifurca* males to become sexually mature, their testes increase from 11.95 ± 0.25 mm ($n=25$) to 68.89 ± 0.44 mm ($n=25$), at which size they occupy more than half the abdominal cavity. Using the correlation curve between testis and sperm length³, we estimated the sperm length in 30-day-old *D. bifurca* males to be 58.36 mm, consistent with the direct measurements of Pitnick *et al.* (58.29 ± 0.66 mm, $n=3$)².

Drosophila bifurca displays an intriguing and unique way of offering sperm to the females which we call the 'pea-shooter effect'. Males transfer to females huge 80- μ m-wide spermatid pellets, each made of a single 6-cm-long gamete (see figure, upper panel). Uniquely, these sperm are transferred one after another. This is made possible by the genital tract in *D. bifurca* males narrowing subterminally in a spiral duct which seemingly acts as a bottleneck constraining mature sperm to disentangle from one another, separate and roll up. The very end of the testicular tract is a straight tube, 'the pea shooter', where monospermatic pellets are stored in single file (lower panel). During each short mating, lasting 374.33 ± 14.63 s ($n=18$), 23-day-old males offer 25.61 ± 1.86 ($n=18$) such monospermatic pellets to females, around four times less male gamete supply than in *D. hydei* whose sperm are three times shorter and remain

mixed during transfer¹. *Drosophila bifurca* males do not transfer all the monospermatic pellets that they manufacture (106.15 ± 30.35 ; $n=26$ per seminal vesicle). Curiously, the amount of monospermatic pellets offered corresponds almost exactly to the number of spermatids per cyst (that is, 24).

The mechanism evolved by *D. bifurca*



Single giant sperm pellets in *Drosophila bifurca*; above, two giant sperm disentangled and rolled up, head in blue (arrow), flagellum in green (methods as in Bressac *et al.*⁶; scale bar, 50 μ m); below, giant sperm pellets ordered in single file in the outer part of the seminal vesicle and released one after another ('pea-shooter effect'), phase contrast (scale bar, 80 μ m).

could be a sophisticated way to fragment the sperm supply within the seminal vesicles and hence facilitate flexible sperm ejaculate allocation. It may enable males to adjust sperm offer, depending on prevailing conditions. This may limit rapid sperm exhaustion in males in a highly promiscuous reproductive system.

Alternatively, transfer of single monospermatic pellets in succession may represent an efficient way of transferring extremely long sperm to females safely. The reason for manufacturing giant sperm is unclear; they may serve as a blocking device, reducing sperm competition by purely mechanical means⁴. This could not

satisfactorily explain why females have evolved co-adapted giant tubular storage organs (73 ± 1.57 mm; $n=25$) within which sperm are stored elongated. A way to counter this difficulty would be to assume that the evolution of co-adapted giant sperm, testes and female storage organs results from a maternal–paternal genetic conflict⁵. Giant sperm could, for instance, result from a coevolutionary arms race between the female and male genital tracts, the paternal genome trying to produce as long sperm as possible to saturate the storage organs of females and thereby limit sperm competition, and the maternal nuclear genome trying to remove the effect of such elongation to promote sperm competition.

Another possibility could be some post-fertilization paternal investment¹ from which females would also gain some benefit. Relevant to this is the seemingly controversial consideration that only incomplete fragments enter the eggs in giant sperm species². Assuming giant sperm constitute a 'direct paternal legacy to the embryo, which, in contrast to any male-derived nuptial gift, cannot be minimized by female remating'¹, females would nonetheless divert for their own benefit a part of the giant sperm otherwise used for the present fertilization (a resource for future offspring sired by subsequent males). These alternative views, however, remain purely speculative, and a difficulty in understanding the problem of why giant sperm have evolved can result from the fact that the various species may obey different selective rules. Sperm disentangling occurs in *D. bifurca* but not in any other giant sperm species so far studied. The existence of monospermatic pellets offered one after another indicates that there are in fact few physiological constraints limiting the range of allocation strategies available to males. It is certainly a quirk of evolution that these sperm mimic the size and form of ova.

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Scientific Correspondence

Scientific Correspondence is intended to provide a forum in which readers may raise points of a scientific character. Priority will be given to letters of fewer than 500 words and five references.

A three-dimensional self-consistent computer simulation of a geomagnetic field reversal

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A three-dimensional, self-consistent numerical model of the geodynamo is described, that maintains a magnetic field for over 40,000 years. The model, which incorporates a finitely conducting inner core, undergoes several polarity excursions and then, near the end of the simulation, a successful reversal of the dipole moment. This simulated magnetic field reversal shares some features with real reversals of the geomagnetic field, and may provide insight into the geomagnetic reversal mechanism.

A FUNDAMENTAL goal of geophysics is a coherent understanding of the structure and dynamics of the Earth's interior. An integral part of this understanding must be a model of the Earth's magnetic field that reproduces its salient features: a field that is maintained for many magnetic diffusion times, is dominantly dipolar at the surface with a dipole axis that on the average lies close to the geographic axis of rotation, and exhibits secular variation with occasional excursions and reversals. The only plausible candidate for such a model is the dynamo model, in which new magnetic field is continually being generated by the shearing and twisting fluid motions within the Earth's liquid, electrically conducting, outer core^{1,2}. According to ref. 3, Albert Einstein considered understanding the origin of the Earth's magnetic field as being one of the five most important unsolved problems in physics. Today we know that a nonlinear three-dimensional model of the magnetohydrodynamics (MHD) of the Earth's core is needed to explain the structure and the long-term evolution of the geomagnetic field.

There are two principal reasons for this. First, it is widely perceived that linear mathematical analysis, which has provided great insights into basic dynamo processes, is unlikely to contribute as fundamentally to the understanding of the geodynamo because the Earth operates in the so-called 'strong-field' regime. Analytical methods are successful in studying 'weak-field' regimes, in which the magnetic forces can be treated as a perturbation in the dynamics. However, in the strong-field regime the nonlinear magnetic Lorentz force is as large as the Coriolis force and therefore cannot be treated as a perturbation. A nonlinear numerical computation is therefore required to simulate the MHD.

The second reason arises from Cowling's theorem⁴, which shows that a self-sustained magnetic field produced by a dynamo cannot be axisymmetric. But most numerical models of dynamos have been 'mean-field' kinematic models, which require a prescription of the axisymmetric effects of some hypothetical three-dimensional convection and solve for the evolution of only the two-dimensional axisymmetric parts of the magnetic fields⁵. Intermediate mean-field models⁶⁻¹¹ go somewhat further by also solving for the axisymmetric zonal flow and meridional circulation, but still require a prescribed two-dimensional structure of some hypothetical averaged helical flow (the alpha effect²) and usually a prescribed structure for the buoyancy force or for the zonal thermal wind shear it produces (the omega effect¹). They are therefore not self-consistent because the solutions strongly depend on how one chooses to prescribe the alpha and omega structures. The simplest way to generate a three-dimensional

magnetic field is to drive it with a prescribed three-dimensional velocity profile¹²⁻¹⁴; but in this approach the (kinematic) velocity is not a self-consistent convective solution with nonlinear feedback from the Lorentz force. Travelling-wave solutions can also be generated¹⁵ but lack mode-dependent temporal behaviour. Magneto-convection simulations in three dimensions are closer to self-consistency because the time-dependent thermodynamic, velocity, and magnetic fields are all solved in three dimensions with nonlinear feedback¹⁶⁻¹⁹; but the main part of the magnetic field is maintained via boundary conditions. These approaches have been used because of their simplicity and relatively small computing resource requirements; but they are limited because either they do not produce self-consistent solutions of the full three-dimensional MHD equations or, in magneto-convection, they do not produce self-sustaining magnetic fields and are therefore not suitable for studying field reversals. They have been and continue to be extremely useful for providing a relatively cheap way of testing the influence of new physics⁹ or studying secular variation of the field on short timescales¹⁹.

Self-consistent numerical simulations of three-dimensional convective dynamos have been computed in planar (cartesian) geometry to study local dynamo action²⁰⁻²³ and in global (spherical) geometry²⁴⁻²⁹ to study the solar dynamo. Of these, however, the only models that generate magnetic energy an order of magnitude greater than kinetic energy and therefore begin to approach the strong-field regime appropriate for studying the geodynamo are the recent models by St Pierre²³ and Kageyama *et al.*²⁹. In the other models, the generated magnetic energy is several orders of magnitude less than the kinetic energy.

Here we present a fully self-consistent three-dimensional numerical simulation of a convective strong-field dynamo in a spherical shell with a finitely conducting inner core³⁰. A magnetic field is maintained for more than three magnetic diffusion times and has energy at least three orders of magnitude greater than the kinetic energy of the convection that maintains it. The exciting feature that we focus on in this paper is a reversal of the dipole polarity that occurs near the end of our simulation. The simulation required over 2,000,000 computational time steps that, over a period of more than a year, took more than 2,000 CPU hours on a Cray C-90, which is why we have decided to report on our results now instead of waiting for our simulation to span a much longer period of time. With only one reversal simulated we cannot yet say anything about the statistical behaviour of reversals in our model. Some minor changes in our model would also bring it closer to geophysical reality. But because our simulation is self-consistent and maintains a field that resem-

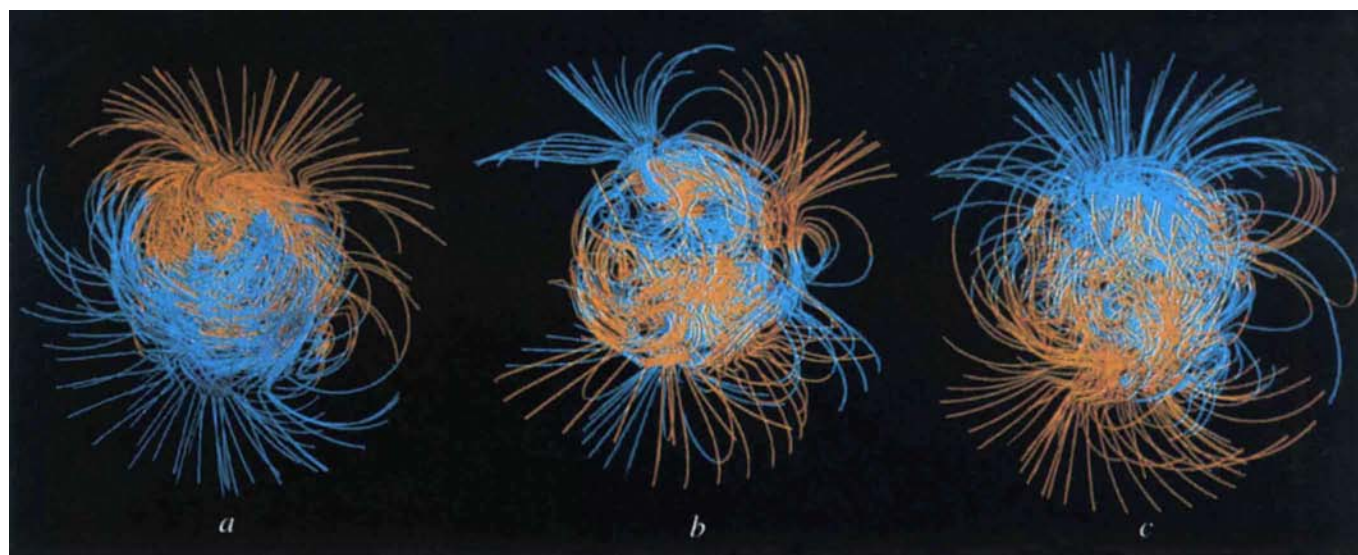


FIG. 1 The three-dimensional (3D) magnetic field structure portrayed through lines of force plotted out to the surface of our modelled Earth. Snapshots are displayed: a, before the reversal (9,000 years before the end of our simulation); b, midway through the transition as seen at the surface (that is, when the axial dipole part of the field at the surface

goes through zero, about 4,000 years before the end); c, after the reversal (at the end of our simulation). Lines are yellow (blue) where the radial component of the field is directed outward (inward). The rotation axis is vertical. One hundred lines of force are plotted in each snapshot, so the relative field intensity is not represented in this figure.

bles the Earth's in many respects, we believe that it provides a plausible description of the geomagnetic field and the way it reverses.

Model description

The numerical model (G.A.G., manuscript in preparation) solves the nonlinear MHD equations that govern the three-dimensional structure and evolution of an electrically conducting fluid undergoing thermal convection in a rapidly rotating spherical shell, our model of the Earth's outer fluid core. A specified heat flux at the inner core boundary (ICB) drives thermal convection in the fluid core. This convection, influenced by the rotation of the core, twists and shears magnetic field, generating new magnetic field to replace that which diffuses away. The field diffuses into a solid, electrically conducting, inner core providing magnetic torque between the inner and outer cores. Magnetic torque also exists between the outer fluid core and a solid mantle above through a thin conducting layer at the core mantle boundary (CMB). The rest of the mantle is assumed to be an insulator, so the field above this layer is a source-free potential field. Time-dependent rotation rates of the solid inner core and solid mantle are determined by the net torques at the ICB and CMB, respectively.

The model prescriptions³⁰ (mass, dimensions, rotation rate and material properties) are Earth-like, except the applied heat flux at the ICB, which is somewhat higher to compensate for the lack of compositional buoyancy sources in this version of our model, and the viscosity of the fluid core, which owing to computational limitations was chosen to be larger than the Earth's. But because the viscous forces in our model (outside the thin viscous boundary layers) are already about six orders of magnitude smaller than the Coriolis and Lorentz forces, our solution is probably in the correct asymptotic regime for the Earth. In comparison with two other strong-field dynamos, Kageyama *et al.*²⁹ assume that the convecting fluid is a compressible gas with a viscosity more than two orders of magnitude greater than our viscosity (when scaled appropriately, using the Earth's rotation rate and core radius), and St Pierre²³ uses a viscosity slightly larger than in our model.

The electrical conductivity of the solid inner core is probably much the same as that of the fluid outer core but, in our initial test calculations (those done before the simulation we are reporting on here), we assumed for simplicity an insulating inner core. The result was a chaotic magnetic field that, unlike the Earth's, reversed its dipole polarity roughly every thousand years. Hollerbach and Jones⁹ then demonstrated, in their two-dimensional mean-field dynamo model, that a finitely conducting solid inner core provides a degree of stability to the magnetic field. Their alpha-omega dynamo generates a field that never reverses, although the magnetic energy undergoes periodic changes. The lack of field reversals in their solution is probably because of the particular prescription of their alpha and omega driving terms that are held constant in time. But the reversal inhibition they demonstrated and the reversal mechanism they suggested motivated us to include a finitely conducting inner core in our three-dimensional convective model. Again, in comparison, Kageyama *et al.*²⁹ do not have a finitely conducting inner core and therefore have no magnetic coupling between their outer and inner cores, and the model by St Pierre²³ is planar.

Our present solution, with a finitely conducting inner core, spans >40,000 years, more than three magnetic diffusion times, with no indication that it will decay away, which is suggestive evidence that our solution is a self-sustaining convective dynamo. The solution begins with random small-scale temperature perturbations and a seed magnetic field. After an initial period of adjustment (~10,000 years) during which the dipole part of the field gradually becomes dominant, our time-dependent solution maintains its dipole polarity until near the end of the simulation, when it reverses in little more than 1,000 years and then maintains the new dipole polarity for roughly the remaining 4,000 years of the simulation.

Field structure during the reversal

To illustrate the magnetic field reversal mechanism, we display the change in the structure of the field during the last 9,000 years of our simulation with three snapshots in each of Figs 1–4. The three-dimensional field structure is portrayed in Fig. 1 via lines of force at 9,000 years before the end of our simulation (Fig.

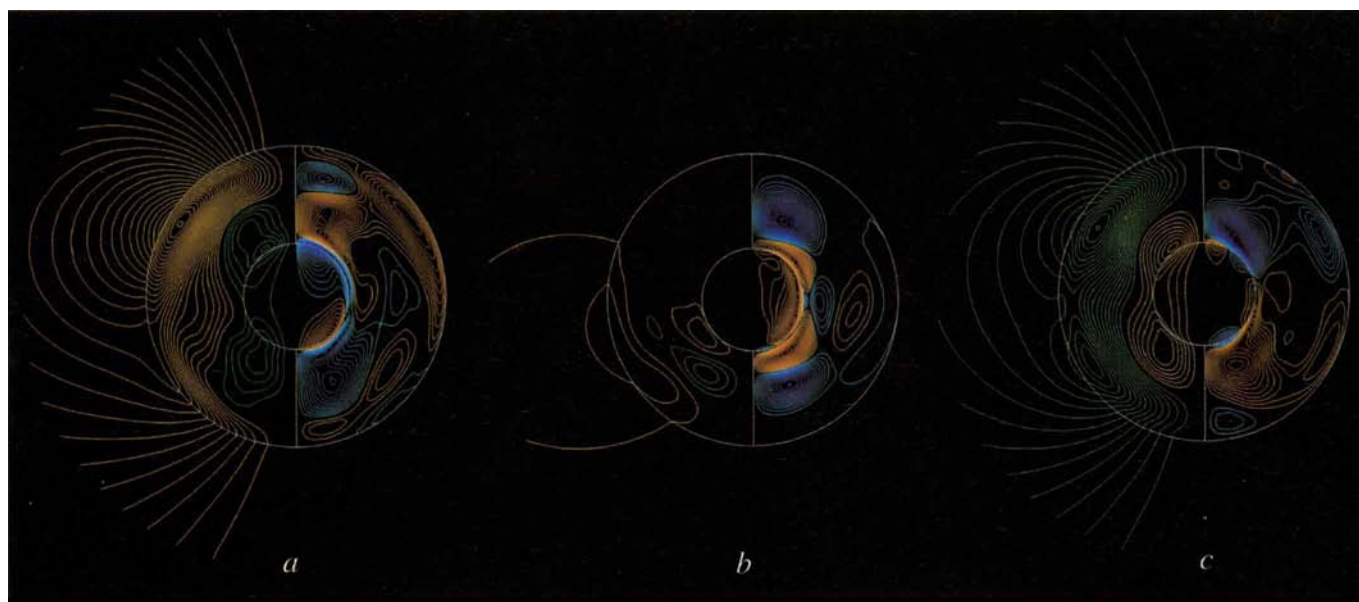


FIG. 2 The longitudinal average of the 3D magnetic field displayed with contours of the toroidal (east–west) part of the field plotted in the right hemispheres and lines of force of the meridional (poloidal) part of the field plotted in the left hemispheres. Snapshots are displayed: a, before the reversal (9,000 years before the end of our simulation); b, midway through the transition as seen at the ICB (that is, when the axial dipole part of the field at the ICB goes through zero, about 5,000 years before the end); c, after the reversal (at the end of our simulation). The north

(south) geographic pole is at the top (bottom) of each plot. The outer circular boundary is the core–mantle boundary and the inner circular boundary is the inner core boundary. Red (blue) contours are eastward (westward)-directed toroidal field. Green (yellow) lines of force are clockwise (anticlockwise)-directed poloidal field that are plotted out to the surface. Note that for the series of plots in this figure the relative field intensity is represented in terms of the number of toroidal field contours and the density of poloidal field lines.

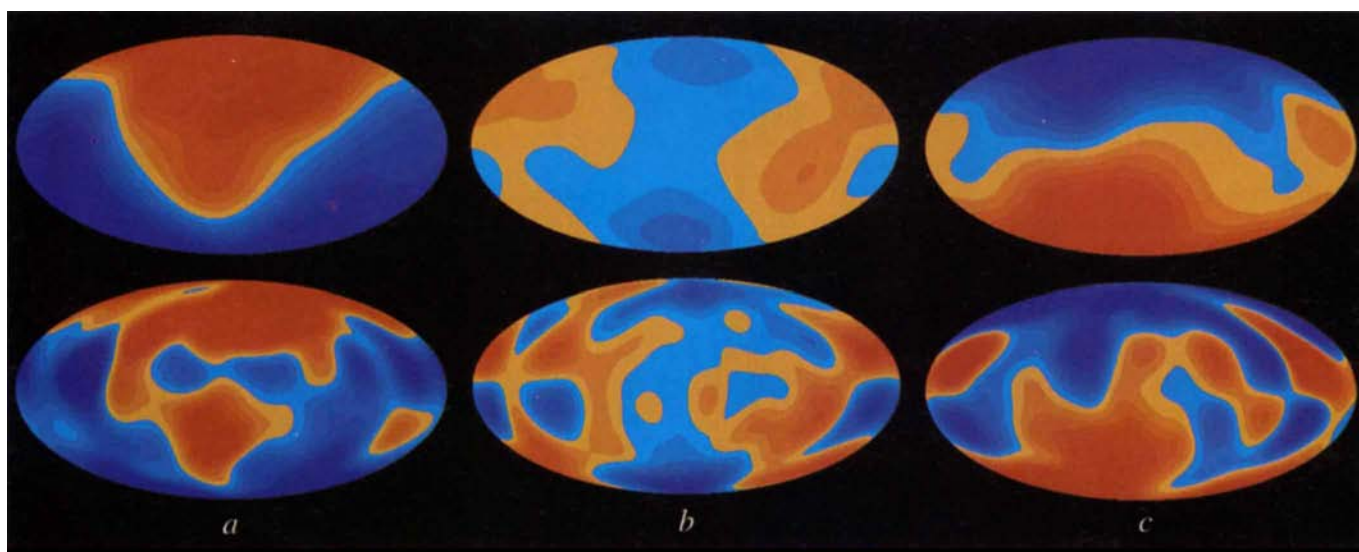


FIG. 3 The radial component of the magnetic field plotted in a Hammer projection of the core–mantle boundary (lower plots) and of the surface (upper plots). Snapshots a, b and c are displayed at the same times as those in Fig. 1. Red (blue) contours represent outward (inward)-directed

field. The relative intensities at the different times are also reflected in the colour contours. But the field intensity at the surface has been multiplied by a factor of ten to obtain colours in the surface plots that are comparable to those in the CMB plots.

1a), at roughly the middle of the polarity transition as seen at the surface (~4,000 years before the end; Fig. 1b), and at the end of our simulation (Fig. 1c). We begin plotting the lines of force at the surface of our modelled Earth. They penetrate inward through the insulating mantle and then into the outer fluid core where the field is generated. The transition from the relatively smooth structure of the potential field outside the core

to the much more complicated and intense field structure inside the core is quite striking. The maximum field intensity usually occurs near the ICB and is typically between 30 and 50 mT. The field at the surface has a dominantly dipolar structure before (Fig. 1a) and after (Fig. 1c) the reversal, with the dipole axis nearly aligned with the rotation axis, which is vertical in Fig. 1. During the polarity transition (Fig. 1b) the field structure at the

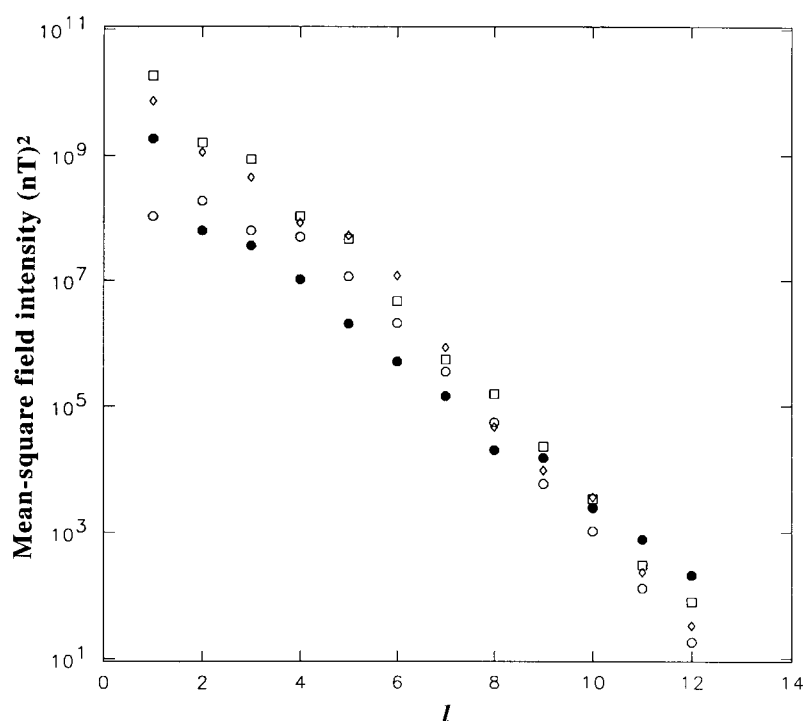


FIG. 4 The mean-square field intensity over the surface plotted as $(l+1) \sum_m [(g_l^m)^2 + (h_l^m)^2]$, where g_l^m and h_l^m are the traditional Gauss coefficients for the potential field outside the core, and l and m are the degree and order, respectively, in the spherical harmonic expansion. Spectra are displayed at the same three times as those in Figs 1 and 3: squares before, open circles during, and diamonds after the reversal. The spectrum for the present-day Earth, based on MAGSAT data³⁴, is plotted as filled circles.

surface is much more complicated and the dipole axis passes through the equatorial plane.

To illustrate the field structure within the inner and outer cores more easily, we plot in Fig. 2 the longitudinal average of the three-dimensional field at 9,000 years before the end (Fig. 2a), at roughly the middle of the polarity transition as seen at the ICB ($\sim 5,000$ years before the end; Fig. 2b), and at the end of our simulation (Fig. 2c). The right side of each plot shows contours of the east-west (toroidal) part of the field; the left side shows lines of force of the meridional (poloidal) part of the field. There are typically two main toroidal field concentrations, one in each hemisphere, in opposite directions and usually inside the imaginary cylinder tangent to the inner core where large zonal flows shear poloidal field, so generating toroidal field. Toroidal field also diffuses into the inner core from the ICB, where it is generated when poloidal field is sheared by the inner core as it moves differentially with respect to the fluid just outside the ICB.

The longitudinally averaged poloidal field (left sides of Fig. 2) typically has two dipolar polarities: one in the outer part of the fluid core, which is also the dipole polarity observed at the surface, and the opposite polarity in the inner part of the fluid core and the inner solid core. Poloidal field is generated by helical flow that twists toroidal field; in the Northern Hemisphere, for example, the time-dependent helicity of the flow (that is, the correlation between velocity and vorticity) is usually right-handed and much larger inside the 'tangent cylinder' and left-handed outside³⁰. This illustrates one of the limitations of models that require, unlike ours, a prescription of the fluid flow instead of solving for it self-consistently. For example, the helical flow structure prescribed by Hollerbach and Jones⁹ has no radial or time dependence and the field has only a single dipole polarity that never reverses. By contrast, it is the interaction of the two dipolar polarities present in our solution that plays an essential role in our reversal mechanism in a way that brings to mind the simple interaction between the two disks of the Rikitake dynamo model³¹.

A movie of our simulation shows how the field in the fluid outer core is continually attempting to reverse its axial dipole polarity on a short timescale (~ 100 years) corresponding to convective overturning but usually fails because the field in the solid

inner core, which can only change on a longer diffusive timescale ($\sim 1,600$ years), usually does not have enough time to diffuse away before it is regenerated at the ICB. It also shows how the axial quadrupole part of the outer field tends to reverse its polarity on a roughly thousand-year timescale, causing a hemispheric (non-reversing dipole) oscillation in the structure and intensity of the outer poloidal field¹⁰ because the sum of an axial dipole and an axial quadrupole results in an enhanced axial poloidal field in one hemisphere and a diminished poloidal field in the other. Once in many attempts the three-dimensional configurations of the buoyancy, flow and magnetic fields in the outer core are right for a long enough period of time for the inner core axial dipole field to diffuse away (Fig. 2b), thus allowing the reversed axial dipole polarity in the outer core to diffuse into the inner core. If this one model is representative of the Earth, it suggests that the strong nonlinear feedbacks in three dimensions and the different timescales of the fluid and solid cores are responsible for the Earth's stochastic reversal record.

When the reversal occurs in our simulation, the energy of the total field in the core is about one-quarter of its typical value. Once the new field polarity has become established, the magnetic energy in the fluid core quickly recovers. During the transition, eastward and westward toroidal field are alternately generated several times in both hemispheres at the ICB before the reversed polarity finally becomes established. Notice how the toroidal field is asymmetric with respect to the Equator before (Fig. 2a) and after (Fig. 2c) the transition but is symmetric midway through the transition (Fig. 2b). In our simulation, the toroidal field reverses first, then the inner poloidal field that penetrates the solid core, and finally, somewhat later, the outer poloidal field that appears at the Earth's surface reverses. This entire process takes (depending on how one defines the beginning and end of the reversal) a little more than 1,000 years, roughly the characteristic magnetic diffusion timescale for the inner core.

Figure 3 shows snapshots of just the radial component of the field plotted at the CMB (lower plots) and at the surface (upper plots) at the same three times as those depicted in Fig. 1. Because the dipolar part of the pattern decays the most slowly with radius, its contribution is greater at the surface than at the CMB. The dipole parts of these fields are nearly axial before (Fig. 3a) and after (Fig. 3c) the transition and equatorial midway through

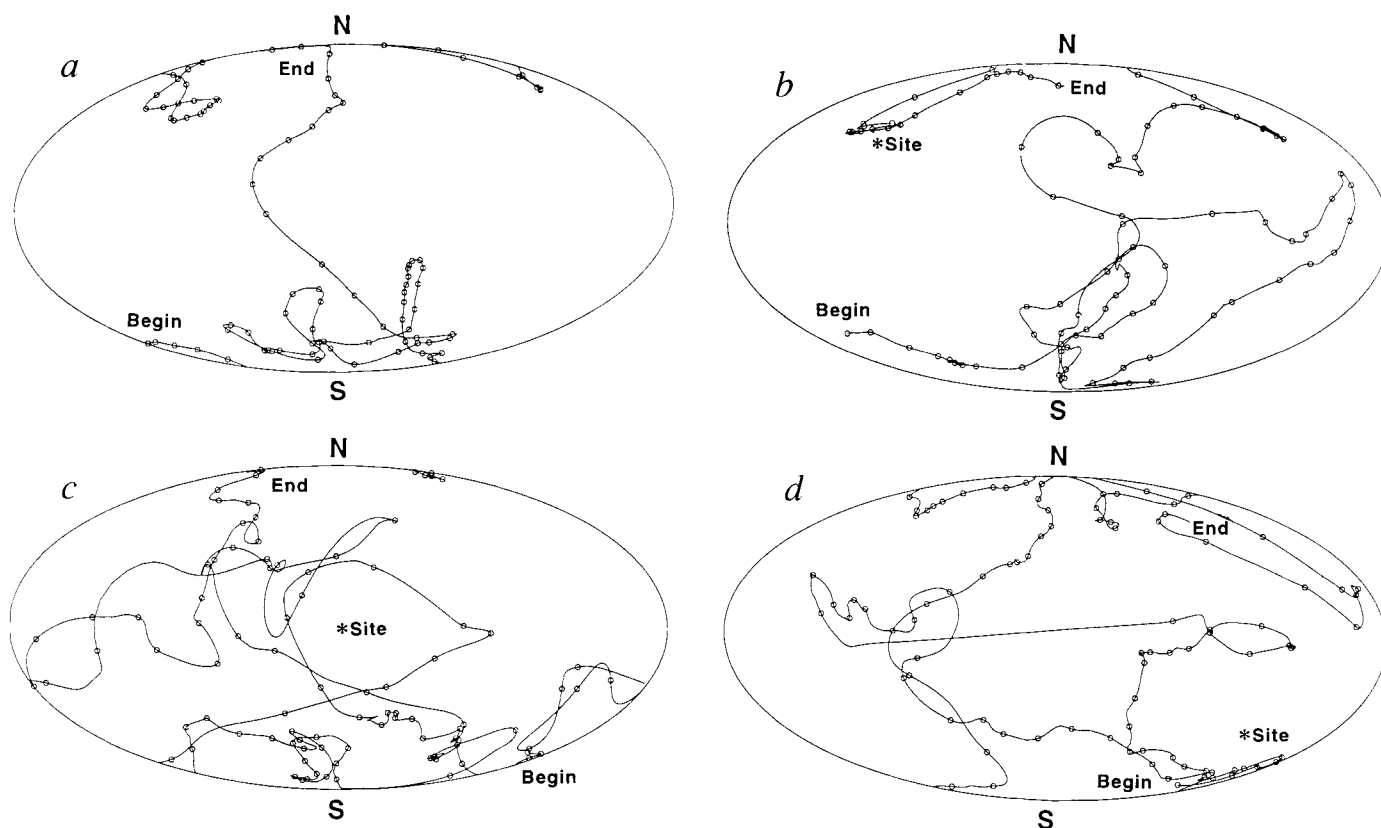


FIG. 5 The true geomagnetic pole (a) and the virtual geomagnetic poles (b–d) computed using the field directions at three surface sites: a, $+40^\circ$ latitude and 60° longitude, b, 0° latitude and 180° longitude, and

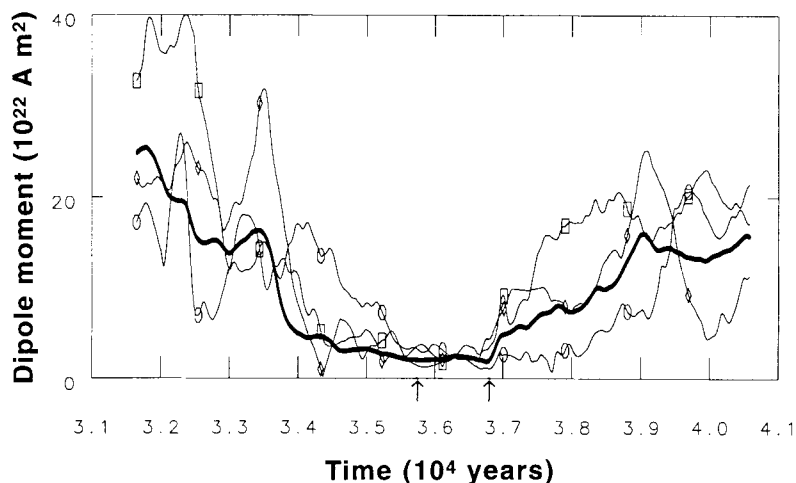
c, -40° latitude and 300° longitude. The pole, with the inward-directed field, is plotted in a Hammer projection of the surface with markers at 100-year intervals during the last 9,000 years of our simulation.

the transition (Fig. 3b). But the dipole part of the field decreases more during the transition than the other modes do; therefore the equatorial dipole part of our transitional field is less dominant than the axial dipole part typically is before and after the transition. For example, the large radial fields at the poles in Fig. 3b are the result of a relatively strong quadrupole mode.

The CMB plots of Fig. 3a, c are qualitatively similar to the Earth's present field projected onto the CMB³² with multimode contributions and several flux concentrations. The maximum intensity of the radial component of the field at the CMB in Fig. 3a, c is ~ 3.0 mT; the maximum intensity in Fig. 3b is 0.8 mT. These values can be compared with the maximum intensity of the Earth's present radial field at the CMB (ref. 32), which is ~ 1.0 mT.

Our simulated field structure at the surface can be compared with the Earth's present surface field structure by plotting the mean-square field intensity over the surface as a function of the spherical harmonic degree, with the traditional Gauss coefficients^{33,34}. The spectra (displayed only out to spherical harmonic degree 12) at the same three times as those used in Figs. 1 and 3 are plotted in Fig. 4. The spectra corresponding to the snapshots long before (squares) and long after (diamonds) the transition show the dipolar ($l=1$) contribution dominant over the quadrupolar ($l=2$) and octupolar ($l=3$) contributions. These two spectra indicate that we could be driving the convection a little too hard because our magnetic energies for the low degrees are higher than those of the present-day Earth^{33,34}, which are plotted as filled circles in Fig. 4. Also, because we have no

FIG. 6 The true dipole moment (heavy curve) and virtual dipole moments (marked by squares, circles and diamonds) computed using the field at the three sampling sites used in Fig. 5 during the last 9,000 years of our simulation. The times indicated are from the beginning of our simulation. The left arrow marks the mid-point in the transition as seen at the ICB, and the right arrow marks the mid-point as seen at the surface.



crustal magnetic fields in our model, the slopes of our spectra do not become shallower with increasing degree above $l=12$ (not shown), in contrast with the Earth's^{33,34}. Our spectrum taken midway through the transition (open circles) shows that the dipole contribution has decreased more than the other modes during the reversal, as is also apparent in Fig. 1. During our entire simulation, magnetic energy is continually being exchanged in a chaotic manner between the modes, especially back and forth between the dipole ($l=1$) and quadrupole ($l=2$) modes on a few-thousand-year timescale. Could the decrease in the Earth's dipole moment over the past 150 years³⁴ be a similar adjustment?

The reversal sampled at different sites

To compare our simulated reversal with the palaeomagnetic reversal record we now describe how our reversal appears as observed locally at different sampling sites at the surface. We have analysed directional plots (declination and inclination) of our surface field at 50 sites distributed over the surface spanning the last 9,000 years of the simulation. These plots (not shown) indicate that the evolution of the local field direction depends greatly on the sampling location; but they all show that a reversal has occurred. By monitoring the rate of change of our surface field direction at these 50 sites we find the largest rate (at one site, at one time) to be 0.1° per day. But most sites have a maximum rate of $\sim 0.01^\circ$ per day during the reversal, which is larger than the rate observed in the secular variation of the present-day geomagnetic field³⁴ but much less than the impulse of 6° per day inferred from the Steens Mountain record³⁵, the largest rate seen in the palaeomagnetic reversal record.

By knowing the local direction of the surface field at a sampling site (that is, declination and inclination) and assuming the field to be purely dipolar, one can determine the geographical location of the virtual geomagnetic pole (VGP)³. In our model we can isolate just the dipolar part of the field (that is, spherical harmonics of degree $l=1$) and compute the geographical location of the true geomagnetic pole (TGP), a luxury that palaeomagnetists do not enjoy owing to the relatively sparse set of sampling sites and the uncertainty in correlating the ages of samples from the different sites. The TGP path (Fig. 5a) during the last 9,000 years of our simulation shows several excursions (or aborted reversals)³⁶ occurring at different longitudes before and after the successful dipole reversal occurs. The transition itself, seen in our TGP path, takes only $\sim 1,200$ years (depending on how one defines the beginning and end of the transition) compared with estimates that range from 1,200 years to the more likely $\sim 5,500$ years, seen in the palaeomagnetic reversal record^{35,37,38}.

We have plotted in Fig. 5b–d the VGP paths over the surface during the last 9,000 years of our simulation at three arbitrary sampling sites. Although these VGP paths show that a reversal has occurred, the details are strongly dependent on the sampling site (marked by an asterisk) and do not resemble the TGP path. For example, the long, nearly horizontal, 100-year segment of the VGP path in Fig. 5d corresponds to an 'impulse' that is also seen in the directional plot at the same site but is not seen in the other VGP paths or in the TGP path. In addition, our estimate of the transition time would be significantly longer than 1,200 years if we based it only on these VGP paths.

These plots can be compared with VGP transition paths or patches from the palaeomagnetic record, which some argue occur at preferred longitudes^{36,39–42} and others interpret in other ways^{43–47}. But because the boundary conditions at the ICB and CMB in our present simulation are spherically symmetric, we do not and would not expect to see our excursions and transition occur along preferred longitudes^{41,48,49}, although we do intend to investigate this intriguing issue with modified versions of our model.

If, in addition to measuring the inclination and declination, one measures the field intensity at a given sampling site, the

virtual dipole moment (VDM) of the assumed dipolar field can be computed³. In Fig. 6 we plot the VDM computed with the field at our same three sampling sites during the last 9,000 years of our simulation. Also plotted in Fig. 6 is our true dipole moment (TDM). The VDMs provide crude approximations to the TDMs; they correctly reflect the general decrease of the TDM before and during the transition and the recovery after the transition, which is similar to what is seen in palaeointensity records³. An asymmetric 'saw-tooth' pattern is also seen in filtered palaeointensity records⁵⁰ but the typical time constant of the cycles is $\sim 500,000$ years, much longer than the time of our simulation spans. It is interesting that our TDM decreases to nearly its minimum value almost 2,000 years before the mid-point in the transition as seen at the ICB (the left arrow in Fig. 6). It remains low until the mid-point in the transition as seen at the surface (the right arrow in Fig. 6), when it begins its recovery. It is typically between 10×10^{22} and 20×10^{22} A m² during most of the simulation, compared with the Earth's present value of $\sim 8 \times 10^{22}$ A m². It reaches a minimum of 1.6×10^{22} A m² midway through the transition, which is $\sim 10\%$ of its usual value compared with the average drop to 25% seen in plots of the VDM for the Earth³.

The relatively poor correlations in Figs 5 and 6 between the three sampling sites are due to the fact that the dipole contribution in our simulated magnetic field is not always strongly dominant (Fig. 4). This illustrates how uncertain the details of the VGPs and VDMs could be if the Earth's field were not strongly dipolar during its reversals as some palaeomagnetic reversal records suggest^{44,51}.

Model limitations and needed improvements

With these initial results from our self-consistent global three-dimensional simulation of the geodynamo we have attempted to bridge the palaeomagnetic and dynamo modelling communities. But with so far only one reversal simulated, our explanations and conclusions about geomagnetic reversals are speculative. Certainly, more analysis is required to improve our understanding of the geodynamo and its reversal mechanism. In addition, several model improvements, which could alter our results and conclusions, will be required for more realistic simulations in the future. For example, more detailed thermodynamics, including compositional convection, is needed. The viscous, thermal and compositional eddy diffusivities should be variable and anisotropic. The effects of topography at the CMB, heterogeneous heat flux and electrical conductivity at the CMB, and luni-solar precession of the mantle could also provide interesting new insights. □

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Transcription complex stability and chromatin dynamics *in vivo*

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Distant regulatory sequences affect transcription through long-range chromatin interactions. Visualization of transcriptional activity of genes that compete for distant elements, using the globin locus as a model, has revealed the dynamics of chromatin interactions *in vivo*. Multiple genes appear to be transcribed alternately rather than at the same time to generate several messenger RNAs in one cell. The regulator may stably complex with one gene at a time and switch back and forth between genes in a flip-flop mechanism.

MANY genes are dependent for expression on the presence of distant regulatory elements which may be tens of thousands of base pairs away. The five human β -globin genes are arranged in the order of their developmental expression (ϵ -G γ -A γ - δ - β)¹ and all are dependent on the locus control region (LCR) for high-level, position-independent expression^{2–4}. The LCR is located over 50 kilobases (kb) upstream of the β -globin gene and consists of five DNase I-hypersensitive sites (HS)^{3,5–7}. The most important sites in terms of transcriptional activation are HS 2–4 (refs 8, 9 and J. Ellis *et al.*, manuscript submitted), each having a core region of 200–300 base pairs (bp)^{10–14}.

Transcriptional competition between genes^{15,16} is important *in vivo* in determining the pattern of globin gene expression during development^{17–20} (N. Dillon *et al.*, manuscript submitted). All of the genes are in polar competition for the activating function of the LCR at all stages of development, with proximal genes having an advantage over distal genes, suggesting that the LCR interacts directly with the gene(s) by a looping mechanism.

The distal β -globin gene is transcriptionally competent at all stages of development, but its expression is suppressed in embryonic erythroid cells by the LCR proximal ϵ - and γ -globin genes. Expression of the β -gene occurs only after silencing of the ϵ - and γ -genes, presumably by multiple silencing elements in the sequences immediately flanking the genes^{21–23}. In contrast to models proposing that different HS regions of the LCR interact with different genes at the same time²⁴, we have proposed that the LCR HS elements interact to act together or form a holocomplex which then interacts with proximal transcriptionally competent genes via looping (ref. 9 and J. Ellis *et al.*, submitted). The LCR holocomplex model would explain why the activity of the HS is additive and why each of the genes requires all of the HS for full expression. Most important, it would explain the fact that there is balanced competition between the genes for LCR function. A key question in validating this model is whether the LCR is limited to activating only a single gene at a time or, as others suggest, the LCR splits its function²⁴ and

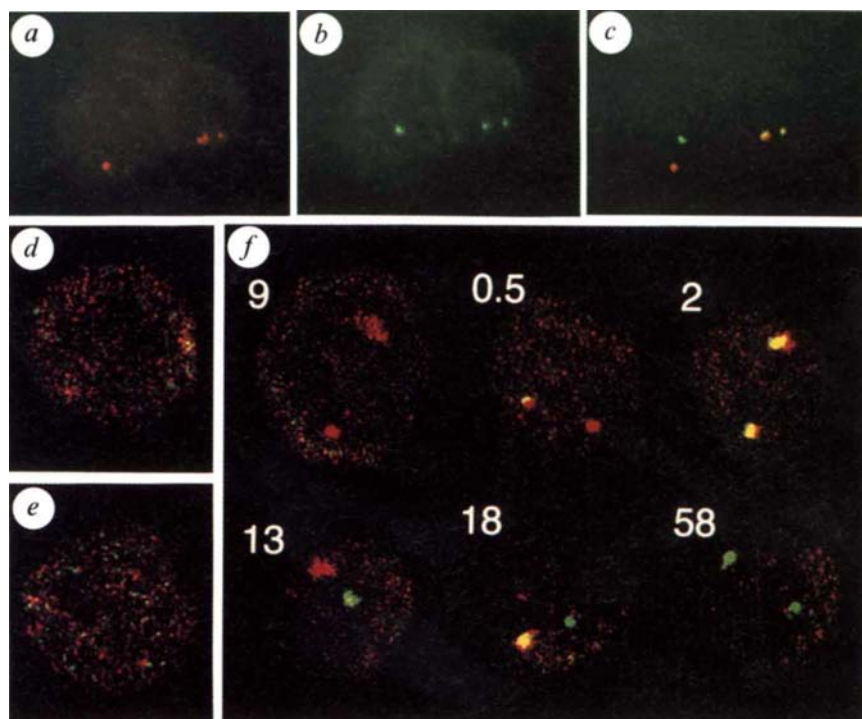
activates several genes simultaneously^{25,26}. We have addressed this question by *in situ* hybridization analysis of the erythroid tissues derived from a transgenic mouse line carrying a single copy of the complete human β -globin locus. The results show that the LCR activates only one gene at a time, indicating that the LCR–globin-gene interaction is monogenic. Furthermore, these interactions are not static, but dynamic, with transcription interactions forming, breaking and reforming in a type of flip-flop mechanism. Finally, the average stability of the LCR–gene interactions was estimated to be of the order of 15–80 min.

Globin primary transcripts

Analysis of γ - and β -globin steady-state messenger RNA levels during switching in the early stages of transgenic fetal liver erythropoiesis (11.5–13.5 d) show that both are detectable in total fetal liver RNA²⁷, with γ -gene expression decreasing and β -globin gene expression increasing. Both γ - and β -globin polypeptides occur in most cells of the transgenic fetal liver^{9,28}, indicating that commitment to γ - or β -gene expression does not occur before the onset of globin transcription. The detection of globin mRNA or polypeptides are not reliable indicators of concurrent transcriptional activity in developing cells owing to the long half-lives of such molecules. Alternatively, primary transcripts are both temporally and spatially associated with the transcriptional event and have short half-lives due to rapid splicing into mature mRNA. The half-life of the mouse β -major globin primary transcript has been calculated to be <5 min in mouse erythroleukaemia cells²⁹. Detection of primary transcripts *in situ* is therefore an accurate indicator of ongoing or very recent transcription. If transcription complexes last longer than the half-life of the primary transcript, two types of signals may occur: single-gene transcription signals or multigene signals. Whereas the explanation for the single-gene signal is obvious, the multigene signal could result from either of two events, namely concurrent transcription from two genes or a recent switch causing a signal overlap between transcription from one

FIG. 1 *In situ* hybridization of transgenic and non-transgenic mouse fetal liver cells. *a–c*, Hybridization of homozygous 12.5-day mouse fetal liver cells (line 72)²⁷ containing two single human β -globin loci; *a* shows the red signal only (Texas red, γ), *b* the green signal only (FITC, β), and *c* the overlay of both after laser confocal microscopy. *d, e*, same as *c*, but in *d* cells have first been treated with RNase; *e* shows results from a non-transgenic fetal liver. *f*, Quantification of the different types of transgenic mouse 12.5-day fetal liver cells. Red (Texas red) represents the γ signal, green (FITC) the β signal, and yellow the combination of both. Scoring was by epifluorescence on a minimum of 400 cells per liver sample and at least two livers. The frequency of occurrence of each cell type is shown as a percentage.

METHODS. 12.5-day fetal livers were disrupted into PBS and the cells fixed onto a poly-L-lysine coated slide in 4% formaldehyde/5% acetic acid for 20 min at room temp.³⁵. Cells were subsequently washed 3 times for 10 min in PBS and stored in 70% ethanol at -20°C . Slides were pre-treated for hybridization by a 0.01% pepsin digestion (5 min, 37°C) in 0.01 M HCl, followed by a short wash in water and a 5-min fixation in 3.7% formaldehyde at room temperature, then washed in PBS, dehydrated in 70, 90 and 100% ethanol steps and air-dried. The hybridization mixture was applied (12 μl per 24×24 mm coverslip) and incubated at 37°C in a moisturized chamber for 12 h. The hybridization mixture contained $0.5 \text{ ng } \mu\text{l}^{-1}$ of each of three or four oligonucleotides (50 nucleotides long) containing a DNP, a digoxigenin or a biotin side chain in the middle and on the 5' and the 3' end of the oligonucleotide (Eurogentec, Belgium) in 25% formamide, $2 \times \text{SSC}$, salmon sperm DNA ($200 \text{ ng } \mu\text{l}^{-1}$), $5 \times \text{Denhardt's}$, 1 mM EDTA and 50 mM sodium phosphate, pH 7.0. The oligonucleotide sequences were derived from the first and second introns of the globin genes and spaced more than 25 nucleotides apart. The coverslip was removed by dipping in $2 \times \text{SSC}$ and the cells were washed three times



gene and the decay of the pre-mRNA (processing) from the other gene.

We have used *in situ* hybridization with gene-specific intron probes to localize human globin primary transcripts in nuclei of individual embryonic and fetal transgenic erythroid cells. The signals appear in the nucleus as fluorescent foci at the location of an actively transcribing gene^{30,31}. The foci do not appear in RNase-pretreated cells or in non-globin-expressing transgenic cells or non-transgenic cells (Fig. 1*d, e*), indicating that the probes specifically detect an intron-containing RNA molecule (the primary transcript). Probe penetration is almost complete as $>97\%$ of the active mouse α -globin genes can be detected (not shown). Heterozygous cells show single foci, whereas homozygous cells show two foci, demonstrating detection of each locus (not shown). To determine whether the LCR can activate the γ - and β -globin genes simultaneously, we performed *in situ* hybridizations with gene-specific intron probes for γ - and β -globin primary transcripts in homozygous day-12 transgenic mouse fetal liver cells (Fig. 1*a–c*). The cell on the right contains primary transcript signals for both γ - (red) and β -globin (green) from each chromosome, suggesting that the LCR can activate both genes simultaneously from a single chromosome, as would be expected for co-transcription. Alternatively, the double signal could be due to an overlap between transcription of one gene and the decay of the pre-mRNA signal from the other gene, as would be expected for single-gene transcription. However, only the latter explanation would also fit the type of cell on the left, which is transcribing only γ -globin from one chromosome and only β -globin from the other. This suggests that the LCR activation mechanism is mono-gene-specific. Considering that these nuclei contain the transacting factors required for both γ - and β -globin transcription, co-transcription would be unlikely if the

in $2 \times \text{SSC}$ at 37°C , followed by a 5-min wash in 0.1 M Tris, 0.15 M NaCl, 0.05% Tween 20. Antibody detection of the labels was essentially as described by Dirks *et al.*³⁵, with three or four amplification steps. Mounting was in DAPI/DABCO:Vectashield (1:1) in glycerol (90%) and stored at 4°C in the dark. Fluorescence was detected by epifluorescence/CCD or laser confocal microscopy. RNase treatment was in 0.1 M Tris, 0.15 M NaCl, $10 \mu\text{g ml}^{-1}$ RNase A for 5 min at room temperature.

γ and β -only cell (left) were to occur frequently, given the high probe penetration ($>97\%$). We therefore quantified the different cell types.

A dynamic mechanism

Day-12 homozygous transgenic fetal liver cells show all possible primary transcript signal combinations of γ - and β -globin (Fig. 1*f*): 58% of globin-transcribing nuclei have only β -globin transcription on both chromosomes and 9% have only γ -globin; the remaining one third of the erythroid cells contain combinations of γ - and β -globin transcription and are therefore involved in the switching process. However, fewer than half (34%) of the chromosomes in these switching cells have both γ - and β -globin signals on the same chromosome. The majority have single gene signals only, suggesting that the LCR interaction is largely mono-gene-specific. Of particular interest is the fact that in $>90\%$ of the switching cells, the two chromosomes are responding differently to the same transacting-factor environment. This suggests a dynamic, continuously changing system in which the individual loci respond stochastically to changes in the factor environment.

Flip-flop

Two forms of such a dynamic process can be envisaged. Either the switch in interaction is progressive from γ to β , or it switches back and forth between the genes in a kind of flip-flop mechanism. γ - and β -globin signals on the same chromosome would then be indicative of a recent switch producing a (brief) period of overlap in which the decaying 'old' primary transcripts are detected in the presence of the newly synthesized transcripts. The length of this overlap period would depend on the half-life of the primary transcript. The progressive switching mechanism

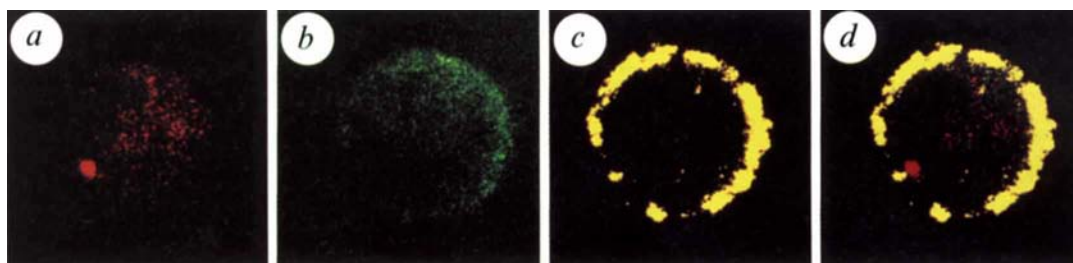


FIG. 2 Detection of γ -globin (Texas red) and β -globin (FITC, green) precursor mRNA in the nucleus and β -globin mRNA in the cytoplasm (Cy3, yellow). The fetal liver was obtained from a transgenic 12.5-day embryo heterozygous for the human β -globin locus. β -globin mRNA was visualized by indirect immunofluorescence with Cy3 of DNP-labelled oligonucleotides. Cy3 emission is also red but at a different wavelength from Texas red and was therefore recorded separately and assigned

an artificial yellow. *a*, Texas red signal (γ pre-mRNA); *b*, FITC green signal (β pre-mRNA)—this image was digitally enhanced to demonstrate the lack of a β transcript, so some bleed-through from the cytoplasmic Cy3 signal appears as a high background; *c*, Cy3 signal (β mRNA). This is a single optical section of the cell from the confocal microscope (0.5 microns); *d* is a composite of *a*, *b* and *c*.

would predict that heterozygous γ -gene-transcribing cells should not have β -mRNA in their cytoplasm, whereas the flip-flop mechanism would result in a proportion of γ (but not β) transcribing nuclei, which contain β mRNA in their cytoplasm.

We therefore added a third probe which specifically recognizes the human β -globin mRNA (exon probe). Heterozygous 12.5-day transgenic fetal liver cells were used, needing only a single chromosome to switch. Figure 2 shows one of many heterozygous cells probed for the γ - and β -globin primary transcripts and cytoplasmic β -globin mRNA. The γ transcription signal is very strong (red in Fig. 2*a*) whereas the β -gene signal (green in Fig. 2*b*) is absent, indicating that the γ gene is currently being transcribed, but the β gene is not. The cytoplasm, however, shows an accumulation of β mRNA, indicating previous transcriptional activity of the β -gene in that cell (Fig. 2*c*). In fact, 40% of the erythroid cells that are transcribing the γ gene, but not the β gene, contain β mRNA. In other words, there is a substantial number of cells that have transcribed β but switched back to γ , an observation that strongly supports a dynamic flip-flop between the genes. Many of the chromosomes with both γ - and β -globin primary transcript signals (Fig. 2) would therefore be the result of flip-flop rather than co-transcription. Thus, the decay time of our signal after a switch becomes important in determining whether most or all of the overlap we observe is due to flip-flop. The 15S mouse β -major globin primary transcript was shown to reach its steady-state level in MEL cells within 5 min²⁹. This indicates that the half-life of the completely intact precursor RNA is considerably shorter as it takes ~ 5 half-lives to reach 97.5% of steady-state level. However, the target of our *in situ* experiments is not only the intact 15S globin primary transcript, but also any other intron-containing RNA molecules such as partially transcribed, partially spliced and excised intron RNAs. We therefore measured the half-life of the intron-containing RNA molecules for mouse β -major globin and human γ - and β -globin in day-12 transgenic fetal liver cells. The results indicate that all three have similar kinetics, reaching steady-state levels simultaneously (Fig. 3). The half-life of the intron is 4–5 min, in agreement with Curtis *et al.*²⁹. Assuming that we can still detect the *in situ* signal after 2–3 half-lives (see discussion), it suggests that the overlap period is 10–15 min.

Replication and switching

One possibility to explain the occurrence of multigene signals when only a single gene is active at any time could be that it is the result of replication of a locus. Homozygous replicating cells (in late S, G2 and M) contain four globin loci in the nucleus and hence a $\gamma\beta$ co-transcription signal could be the result of the γ gene being transcribed from one of the replicated loci, whereas β could be transcribed from its replicated chromatid. This would result in the percentage of $\gamma\beta$ signals being substantially higher

in G2 than in G1 cells. We therefore sorted 12.5-day fetal liver into populations of G1 and G2 cells³² and repeated the hybridizations. Analysis of the G1 and G2 cells shows a distribution and percentage of $\gamma\beta$ signals similar to the total population, with no significant difference between the two purified populations. We conclude that the switch between genes can take place in G1—that is, DNA replication is not required, implying that the LCR–gene interactions are dynamic rather than static. As expected^{33,34}, no transcription signals are observed when the cells are in mitosis.

Co-expression and switching

To extend these results, we characterized the transcriptional status of individual cells at different times of development,

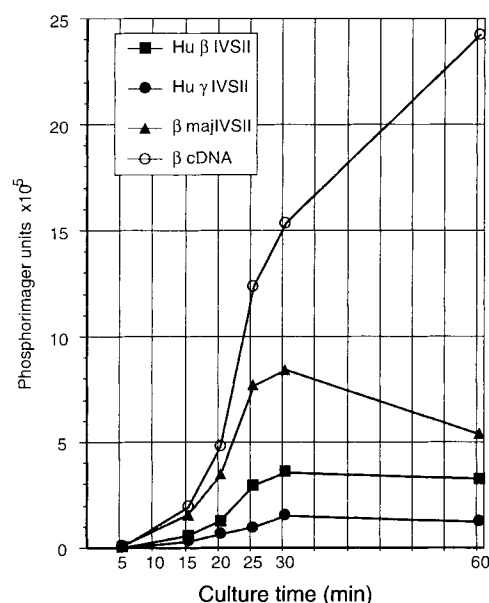


FIG. 3 Incorporation of ^{32}P into globin intron and mRNA sequences. 12.5-day fetal liver cells from heterozygous line 72 were cultured in the presence of ^{32}P for the times indicated, essentially as described²⁹. Half-lives were calculated by comparing the difference in the rates of incorporation between intron and exon sequences. The 4–5-min half-life fits well with the fact that 5 half-lives are required to reach 97.5% of the steady state level.

METHODS. RNA was prepared for 1.5×10^7 cells per time point and hybridized to 5 μg immobilized denatured plasmid DNA as described³⁶. Results were quantified by phosphorimager analysis. Plasmids contained mouse β -major intron II (*Bam*HI/*Pst*I), human β intron II (*Bam*HI/*Ssp*I), human γ intron II (*Bam*HI/*Sac*I) and partial human β -globin cDNA; signals were normalized for relative probe length.

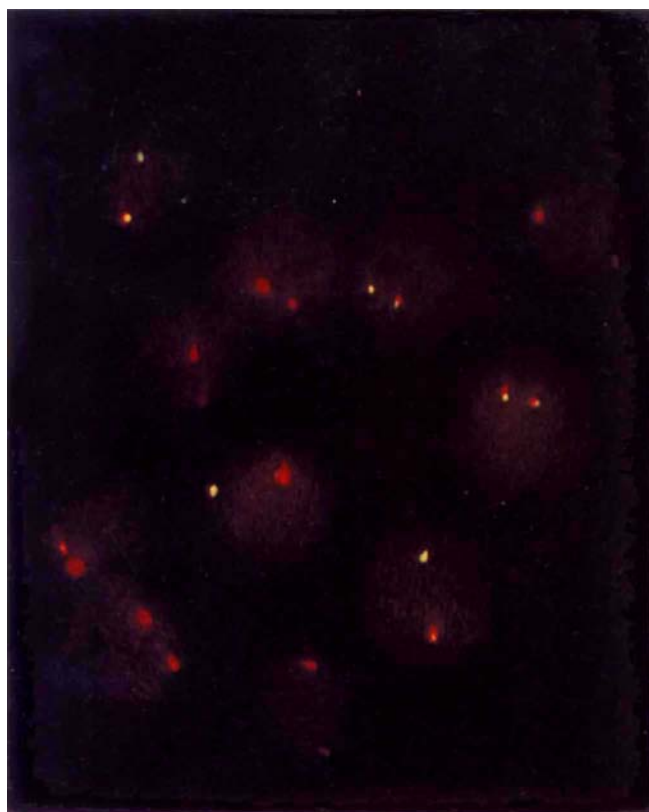


FIG. 4 Simultaneous detection of ϵ (FITC, green) and γ (Texas red) transcription in 10-day embryonic blood. Cells were obtained from a line-72 embryo homozygous for the human globin locus²⁷.

namely day-10 embryonic blood and day-11 fetal liver. During transgenic embryonic erythropoiesis γ - and ϵ -globin expression occurs simultaneously over a period of at least 3 days (days 8–10 of gestation) and nearly all cells contain both ϵ - and γ -globin polypeptides²⁸. We have previously determined the level of expression of the γ - and ϵ -globin genes when present as single genes linked to the LCR and compared this to the level of expression when both are linked together to the LCR. That comparison suggested that the γ and ϵ genes are also in competition for the LCR (P.F., unpublished results), presumably through a looping mechanism (N. Dillon *et al.*, submitted). The circulating embryonic erythrocytes are nucleated and transcriptionally active, and hence were used to determine whether flip-flop could

also be occurring between the ϵ - and γ -globin genes by probing day-10 embryonic erythrocytes with gene-specific intron probes for ϵ and γ (Fig. 4). The results demonstrate (like γ and β in the fetal liver) a heterogeneity in transcriptional signals, including chromosomes with overlapped signals (Figs 4 and 5). We conclude that the LCR also productively interacts with only one gene at a time in peripheral embryonic blood cells and that ϵ and γ are co-expressed via a flip-flop mechanism. The higher incidence of overlapped signals on individual chromosomes suggests that the LCR- ϵ complex is less stable than those of γ and β with the LCR (see discussion).

The analysis of day-11 fetal liver shows that the switching process is a continuously changing system, reflected at the level of the individual cell (Fig. 5). When compared to the day-12 results, there are more $\gamma\gamma$ - and fewer $\beta\beta$ -transcribing cells (27 versus 9% and 35 versus 58%); accordingly, significantly more cells are observed that transcribe γ only from locus and γ and β from the other locus (7 versus <1%). The number of cells transcribing γ only on one chromosome and β only on the other chromosome is very similar (14 versus 13%). These results indicate that the change in the composition of transcription factors during the switch is gradual and slowly changes the affinity between the LCR and the genes towards the adult type LCR/ β -globin gene interaction.

Discussion

We have demonstrated the dynamic nature of the interactions between the human β -globin genes and the β -globin LCR throughout development and shown that the LCR-gene interactions are highly stable. Our data can all be explained by a mechanism in which the LCR activates only a single gene in the locus at any given time. The LCR, although made up of a series of hypersensitive sites, could act as a functional unit or holocomplex that activates multiple genes from the same chromosome via a stochastic mechanism of dynamic rather than static interactions (Fig. 6). Although a low level of co-transcription cannot be excluded such a mechanism cannot account for our observations. Dillon *et al.* (manuscript submitted) have used steady-state measurements of the transcriptional output from mutated loci to demonstrate that the genes compete in a polar manner for the activating function of the LCR. They find that the relative distance of the genes from the LCR affects competition and conclude that the genes are activated by direct interaction with the LCR. Thus two studies, which use entirely different approaches, provide strong evidence of direct interaction between the LCR and single genes by a dynamic looping mechanism of transcriptional activation. It does not show whether some process spreading in a linear fashion along the chromatin is involved in the first activation of the locus. The balance of, γ - versus β -gene transcription would gradually change during a

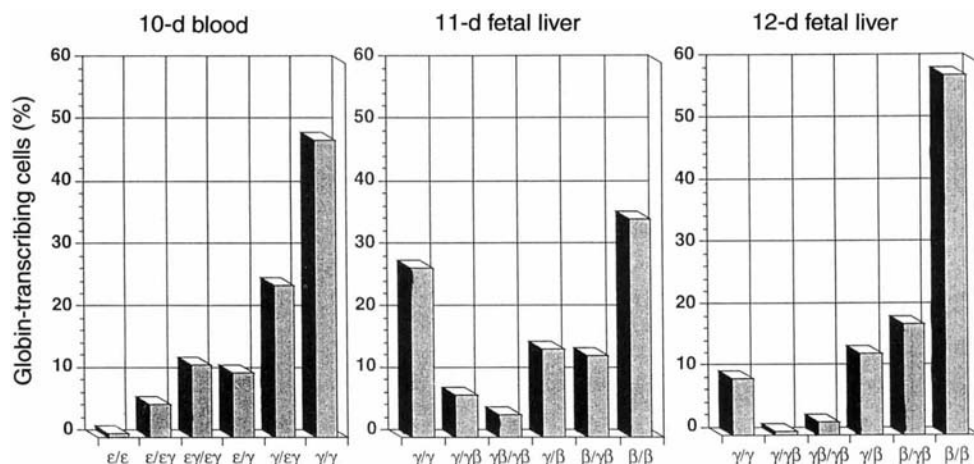


FIG. 5 Bar diagram of the percentages of transcriptional cell types observed at different times of development in transgenic cells homozygous for the human globin locus. A single transcription signal at one of the loci is indicated with a single letter, double signals with two letters. For example $\gamma/\gamma\beta$ is a cell type with a γ signal only on one chromosome and signals for γ and β on the other chromosome. The number of cell types in the population are given as a percentage.

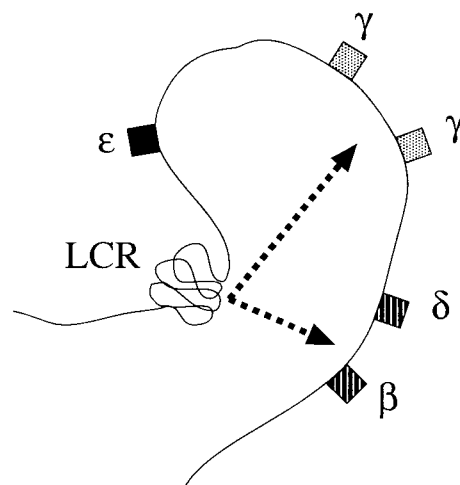


FIG. 6 A model of the interactions of the locus control region (LCR) with the different globin genes through a stochastic looping mechanism. The LCR is indicated as a squiggly line to indicate that the different regions that are hypersensitive to DNase I of the LCR could act together or even form a holocomplex to establish an interaction of the LCR with one of the genes.

switching period as the factor composition changes in the population.

Flip-flop time and complex stability

By scoring the different transcriptional chromosome types in the erythroid nuclei (Fig. 5), it is possible to estimate the flip-flop time, or the average time required for the LCR–gene interaction to change from γ to β (or vice versa), based on the half-life of the intron-containing RNAs and the percentages of different transcriptional states. The half-life determines the decay rate of the signal and thus the overlap time, that is, the length of time both γ - and β -signals are detectable on the same chromosome after a change in LCR–gene interaction. As the cell population is random (so the chromosomes are not synchronized in terms of the γ -to- β switch), the percentage of chromosomes showing a double signal at any particular instant indicates the fraction of the total time in which a chromosome is in the overlapped state. By extrapolation, we can determine the average length of time that a gene is transcribed—that is, the time between flip-flop events. For example, the frequency of overlapped β signals in the population at 12 days is 13% of the total number of β signals. Therefore, on average, 13% of the time in which an individual chromosome transcribes the β gene it will be overlapped by a γ signal. The overlap time can be estimated as 10–15 min on the basis of the intron half-life of 4–5 min and the fact that we can barely detect the precursor RNA when we use one (instead of three or four) oligonucleotides (M.W., unpublished results). As 13% of the β signal is overlap, the total β gene transcription time would be ~ 80 min. (As overlap occurs at the beginning and end of a transcription period, the time in overlap

is 2×10 –15 min. The total transcription time is therefore $100/13 \times 12$ min minus one overlap of 12 min.) Correspondingly, the percentage of overlapped γ signals is 42%, making the average LCR/ γ gene time between flip-flops ~ 16 min. When the results are compared for ϵ , γ and β from days 10–12, several trends become apparent. First, at 10 days, the ϵ signal is overlapped with a γ signal 75% of the time, which suggests that the lifetime of the ϵ -LCR complex is short (about 4 min) and flip-flop occurs more readily. The γ signal is present more often than ϵ at 10 days and is overlapped only 29% of the time, suggesting that the γ -LCR complex is more stable, with ~ 30 min between flip-flops. The average stability of the γ -LCR complex decreases from 10 to 12 days from 30 to 16 min, whereas β increases from 45 to 80 min from 11 to 12 days. Clearly the time between flip-flops decreases for γ but increases for β . Obviously interaction could consist of rapid associations and dissociations of the LCR with a given gene, rather than one stable association. Whatever the mechanism, our data suggest that the continuous presence of the regulatory elements at a gene are required for multiple rounds of initiation of transcription of that gene, rather than being dispensable after the activation of transcription.

In summary, our technique has enabled us to analyse single genes in single cells and allows us to make the conclusion that γ - and β -globin genes are transcribed alternately rather than concurrently. The ability to visualize specifically the transcriptional activity of individual genes in the nucleus provides a powerful tool for studying developmental transcriptional regulation and chromatin dynamics and should provide a more accurate picture of transcriptional regulation compared with conventional *in situ* analysis. □

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Solar-cycle dependence of the Sun's apparent radius in the neutral iron spectral line at 525 nm

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SPACE-BASED observations have established that the Sun's irradiance varies with solar magnetic activity¹⁻⁴. A fraction of this variability arises from the increased area of cool gas associated with sunspots, which decreases irradiance; but on average the blocking effect of sunspots is more than offset by the increased emission from photospheric faculae and other effects of magnetic activity⁵ (although the precise contributions of these opposing effects remain poorly constrained^{6,7}). Here we show that the apparent radius of the Sun, when viewed in the spectral line of neutral iron at 525 nm, also varies in phase with solar magnetic activity. This variation probably results from changes in the temperature profile of the Sun's atmosphere with the solar cycle. If similar behaviour is found for other spectral lines, changes in the apparent radius of the Sun could account for a significant fraction (~20%) of the total irradiance variations.

The Sun is a completely gaseous self-gravitating sphere that does not have a uniquely defined radius. A frequently intended use of the term 'radius' is to denote the material radius and in this context surfaces of constant density probably provide the best definition. This type of radius has to be inferred indirectly. The type of radius we measure here is derived from the drop-off of brightness of the solar disk at its edge and is a function of the temperature and density profiles. The relationship between the material radius and the apparent radius is complex; however, we note that the Sun's energy output is probably more closely related to the apparent radius than the material radius because the former is derived from the emitted radiation whereas the latter depends on the hydrostatic structure.

The Mt Wilson synoptic programme of solar magnetic observations carried out at the 150-foot tower scans the solar disk

every clear day, using the radiation in the neutral iron line at 525.0 nm. As part of this programme, the Sun's apparent radius in this line is determined as an average distance between the image centre and the point where the intensity drops to 25% of its value at the disk centre. We have made an analysis of this database correcting for such effects as scattered light and atmospheric refraction. Although previous analysis of the database⁸ did not find any variation, improvements in the optical system in 1982 reduced instrumentally induced variations and permit us to measure the changes in the apparent solar radius since that time.

Although the variability of the solar output is definitely established, the detailed dependence of the rate of energy output on the level of solar magnetic activity has not yet been measured with enough continuity and precision to establish the correlation throughout the full solar cycle; it is none the less apparent that higher levels of solar activity coincide with greater solar energy output. This increase in total output occurs in spite of the reduction in radiating surface area caused by the dark sunspots. On timescales of days the Sun's output decreases by easily detectable amounts as solar rotation carries sunspots across the visible surface. In spite of this blocking effect, the brightenings associated with features known as plages and faculae in active regions produce an increase in solar output that more than compensates for the blocking effect. A four-component model composed of quiet Sun, magnetic network, faculae and sunspots⁷ has been developed to account for this compensation. Variations in the Sun's radius are not included in this model.

The Mt Wilson system and its method of measuring the Sun's apparent radius have been described by LaBonte and Howard⁸ and we give here only a brief summary. A portion of the image of the Sun is directed into a spectrograph entrance aperture by a servo-controlled coelostat and doublet objective lens at the top of the tower. The position of the solar image is determined by a guiding system carried on a movable frame near the focal plane whose position is determined by precision screws. The magnetogram is built up by scanning the image over the spectrograph entrance aperture in alternating directions, with the primary scan direction adjusted daily to be perpendicular to the Sun's polar axis. The Sun's image is initially positioned off the north or south pole (chosen randomly) and each magnetogram is built up by successive scans in the primary direction. Each scan line begins and ends at a fixed distance off the solar disk.

The intensity of the disk is used to evaluate the scattered light. During each scan the data system continually samples the intensity and circular polarization in two spectral pass-bands that are maintained by servo control to be equally illuminated on opposite wings of the 525.0-nm spectral line. The spectrum is sampled by means of fibre-optic image reformatters that redirect light from the focus of the spectrograph to photomultipliers. Data obtained before 1982 cannot be used owing to a poorly stabilized system of prisms which determined the spectral sampling.

The limb position is determined during the data reduction process from the point where the sampled intensity is 25% of the disk central intensity. Although LaBonte and Howard⁸ mention a limb definition in terms of the point of greatest slope, the positions recorded in the Mt Wilson data archive are based on the fractional intensity definition. This limb position differs fundamentally from a white-light or broadband filter position because the portion of the spectral line that we use arises from near the temperature minimum rather than the photosphere. The presence of facular brightenings from active regions near the solar limb will also influence our limb-position definition. These effects are, however, relevant to the total

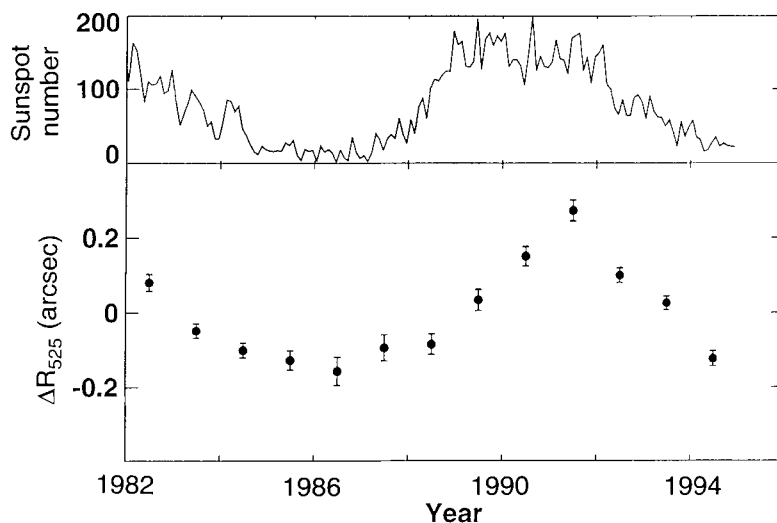


FIG. 1 Time dependence of the solar apparent-radius residuals ΔR_{525} . Lower panel, results of the analysis described in the text. Each point is the annual average of the difference between the apparent radius derived from the observations and the ephemeris radius. The error bars are errors of the mean derived from the daily observations for each year. Upper panel, monthly averages of the International Sunspot Number obtained from the public NOAA databases at Boulder, Colorado.

solar irradiance, which depends on all the radiation emitted from the Sun.

Each clear day 1–25 magnetograms are obtained. Our apparent radius averages are based on just one magnetogram per day and this normally is obtained using a 12-arcsec-square aperture. Occasionally we use a magnetogram obtained with a 20-arcsec-square aperture. These latter are corrected to a consistent size scale by subtracting a fixed offset determined from the whole data set. We present our results in terms of the residuals, which are the differences between the observed solar radius and that calculated from an ephemeris. All observations have been corrected for the effects of scattered light and atmospheric refraction. After these corrections, we have linearly detrended the residuals. We have evaluated the temperature sensitivity of the measured apparent radius and find its influence on the annual averages to be negligible. Our results, in the form of annual average apparent radius residuals, ΔR_{525} , are given in Fig. 1.

Between mid-1981 and 1987 Brown observed the solar radius at the High Altitude Observatory. His report⁹ stated that for this period the annual averages of the diameters differed from one another by less than 0.05 arcsec. The period spanned by our observations and Brown's overlap for 5.5 years near the sunspot minimum. The radius stability reported by Brown is less than the variation we find; but there are three important factors that account for the different results: first, we use light from a spectral line whereas Brown used white light; second, we define the limb as the position where the intensity equals a fraction of the central intensity whereas Brown used a finite Fourier transform definition; and last, Brown only measured the solar equatorial diameter whereas our apparent radius applies to the full solar disk.

Visual measurements of the solar radius have been made with the CERGA astrolabe at l'Observatoire de la Côte d'Azur beginning in 1975 (ref. 10). The most recent published data from this programme¹¹ show variations that are larger than we find and have an opposing phase relative to solar activity. It is likely that the difference between the CERGA results and ours is a consequence of the measurement techniques. Earlier solar-radius measurements have been summarized by Gilliland¹².

Our measurement is distinct from those by Brown and those made with the CERGA astrolabe in that all portions of the solar circumference are weighted equally in our measurement, so we conclude that within spectral lines the radiating area of the Sun is increased at solar maximum. If this translates to a change in luminosity, it would produce a change of 0.08%; a substantial fraction of the observed luminosity variation of $\sim 0.1\%$. But because only a fraction of the Sun's spectrum participates in this radius variation, the effect on luminosity is less. We can estimate the fraction of the Sun's radiation that shows a radius variation similar to what we have observed from the fraction of the emitted energy that comes in spectral lines. We note first that our 0.014-nm-wide sample of the 525.0-nm line is nearly as broad as the whole line: our observations are not confined to the deepest portion of the line.

Although a careful analysis will be needed to determine precisely the fraction of the Sun's radiation that participates in the apparent radius variation, we can make a rough estimate from solar spectral atlases¹³ combined with the solar spectral irradiance¹⁴. Our estimate is based on three broad spectral bands. We estimate the fraction of the total solar irradiance contributed by each band f_i and within each band a fraction ϕ_i of the spectrum that is occupied by lines similar to 525 nm. Assuming that these lines have the same apparent radius change as 525 nm the total irradiance will change by $0.08(\sum_i \phi_i f_i)\%$. The first band blueward of 450 nm is nearly uniformly covered with lines, so $\phi_1 \approx 1.0$. This band contributes 13% of the total irradiance, so $f_1 \approx 0.13$. The second band from 450 nm to 550 nm has $\phi_2 \approx 0.2$ and $f_2 \approx 0.11$. The third band from 550 nm into the infrared has $\phi_3 \approx 0.08$ and $f_3 \approx 0.76$. The sum of the three contributions yields $\sum_i \phi_i f_i \approx 20\%$. We estimate that this number

could be between 15% and 30%. Thus we estimate that the variations in the apparent radii of the Sun's disk at all wavelengths will contribute between 0.013% and 0.025% variations in total irradiance. This is an important fraction of the observed variation of 0.1%. Because a large fraction of the solar spectrum in the blue is affected by spectral lines, our observed apparent radius change can be verified with other systems by using a broadband filter that selects the radiation shortward of 450 nm.

In a related area, solar oscillation frequencies are observed to be dependent on the solar cycle, with those frequencies near the atmospheric cutoff showing the greatest increase near solar maximum^{15,16}. Our observations of a change in the apparent solar radius could assist in the interpretation of the frequency variations because the most probable interpretation of our result is in terms of a change in the temperature profile of the solar atmosphere. If there is an increase in temperature in the upper atmosphere as is suggested by our observations, the higher-frequency oscillations, which are most influenced by the atmospheric cutoff frequency, should show the largest frequency increase, as observed. $\square$

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Enhancement of the dielectric constant of Ta₂O₅ through substitution with TiO₂

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MICROELECTRONICS research is in large part driven by the demand for smaller components with enhanced performance. For capacitive components, which form the basis of many memory devices, the dielectric constant limits the degree of miniaturization—a limit that is now being approached for the materials currently in use. For this reason, exotic compounds with high dielectric constants, such as barium strontium titanate, are being widely investigated¹. But such materials invariably incorporate chemical elements foreign to current microelectronics fabrication procedures, and must pass extensive compatibility tests before they can be used commercially. From a compatibility point of view, tantalum oxide, Ta₂O₅, is considered more promising^{2–5} (although its dielectric properties are more modest), and it is known to form high-quality

thin films in conventional fabrication processes. Here we show that the dielectric constant of Ta_2O_5 can be increased by nearly a factor of four—from 35 to 126—through the addition of 8% titanium oxide, TiO_2 . The minimum area of capacitive components prepared from this material should be reduced by the same factor, and as both tantalum and titanium are compatible with fabrication processes currently in use, the material shows great promise for future microelectronics applications.

Ceramic samples in the Ta_2O_5 – TiO_2 chemical system were made by standard ceramic processing techniques. High-purity Ta_2O_5 and TiO_2 were first mixed in the appropriate molar ratio, mechanically ground, and fired for several nights in dense Al_2O_3 crucibles in air between 1,350 and 1,400 °C with intermediate grinding. The powders were then pressed into 0.5-inch diameter pellets (typically ~0.125 inch thick) and fired on powder of their own composition for 16–24 hours, again in air, at 1,400 °C, and cooled to 1,000 °C at 200 °C h⁻¹ before the furnace was turned off. The pellets as made were near 90% theoretical density, sufficiently dense for the present purposes. Surface of the pellets were than sanded smooth and 1:1 mole ratio Ga:In alloy solder was applied as electrodes. Measurements of the dielectric constant (ϵ_r) and dissipation factors between –20 and 60 °C were made at 100 kHz and 1 MHz with the use of an HP4192A impedance analyser.

The dielectric data for the 1-MHz measurements are summarized in Table 1: the results at 100 kHz are similar. The dielectric constant, dissipation factor, total variation in dielectric constant, and temperature coefficient of dielectric constant (Q_ϵ) in the –20 to 60 °C temperature interval are tabulated for each composition studied. Particular attention was paid to the compositions very close to Ta_2O_5 , as that is where the greatest enhancement of ϵ_r occurs. The dielectric constant is seen to peak strongly at 126 when TiO_2 substitution reaches 8%, as illustrated in Fig. 1. Although ϵ_r for TiO_2 is larger than that for Ta_2O_5 for a wide range of Ta_2O_5 – TiO_2 compositions, the greatest advantages of increased ϵ_r occur between ~95% and 85% Ta_2O_5 .

The temperature-dependence of ϵ_r at 1 MHz for the polycrystalline pellet of Ta_2O_5 : TiO_2 composition 0.92:0.08 is shown in Fig. 2. The last column in Table 1 presents Q_ϵ at ~20 °C for all materials studied. It is seen that the increased dielectric constant that results from TiO_2 substitution of Ta_2O_5 is accompanied by a significant increase in Q_ϵ . The material with the highest ϵ_r has a Q_ϵ about five times that of Ta_2O_5 . This ratio may or may not hold for the same materials in thin-film form. The temperature variation of ϵ_r for some of the materials is illustrated in Fig. 3 which shows that materials near the highest ϵ_r have similar Q_ϵ , strongly favouring the high- ϵ_r material (0.92:0.08) for applications. It also shows that an enhancement of ϵ_r by a factor of 2 over Ta_2O_5 , with lower Q_ϵ than the 0.92:0.08 material, is possible for compositions near 85% Ta_2O_5 . The composition region

TABLE 1 Dielectric properties of bulk polycrystalline Ta_2O_5 – TiO_2 ceramics measured at 1 MHz

Composition				Total change in ϵ_r , –20 to +60 °C (%)	Q_ϵ † (p.p.m. per K)
Ta ₂ O ₅	TiO ₂	ϵ_r at 20 °C	D^* at 20 °C		
1.00	0.00	35.4	0.006	4.8	600
0.98	0.02	20.3	0.016	7.6	950
0.96	0.04	46.6	0.038	20.6	2,580
0.94	0.06	94.0	0.016	22.5	2,810
0.92	0.08	126.2	0.010	23.4	2,930
0.90	0.10	97.8	0.026	24.3	3,040
0.875	0.125	88.6	0.008	16.9	2,110
0.85	0.15	69.1	0.008	13.0	1,620
0.80	0.20	59.4	0.009	11.4	1,420
0.70	0.30	57.6	0.021	12.1	1,510
0.60	0.40	42.2	0.009	9.7	1,210

D is dissipation factor ($=\tan \delta$).

† Total change in ϵ_r (in p.p.m.)/80.

between approximately 94% and 88% Ta_2O_5 yields the best materials if ϵ_r value is the primary consideration. At an optimal Ta_2O_5 : TiO_2 composition of 0.92:0.08 the new material is likely to be processable with very similar conditions to those currently employed to make pure Ta_2O_5 films.

Table 1 also shows the measured values of the dielectric dissipation (D) at 1 MHz. The values of D in Table 1 represent only an estimate of the upper limit of the intrinsic material dissipation, as the effects of series and parallel resistances due to measurement configuration to the measured values of capacitance and dissipation have not been modelled⁶. Unlike the other dielectric data, D does not systematically change with composition. This suggests that the loss values may be dominated by uncontrolled parameters in the present processing procedure, such as the presence or absence of oxygen vacancies, well known to occur in titanium-based oxides. In any case the dissipation factor measured for the 0.92:0.08 composition is not more than double that measured under our conditions for pure Ta_2O_5 , and may actually be of the same magnitude as that of Ta_2O_5 in properly processed materials, as indicated by the lower D for other materials in the table. Figure 4 shows the frequency dependence of the capacitance and loss ($\tan \delta$, dissipation factor) of a polycrystalline pellet of 0.92 Ta_2O_5 –0.08 TiO_2 at 30 °C, in the frequency range 1 kHz to 10 MHz. The data show no strong dielectric dispersion and thus the new material can be expected to display acceptable switching-time characteristics at the frequencies appropriate for dynamic random-access memories. The apparent increase in $\tan \delta$ at the highest measurement

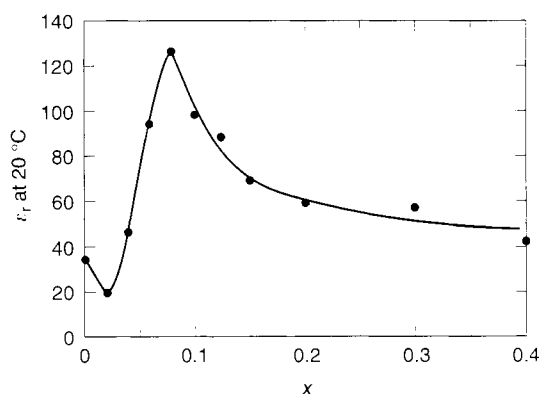


FIG. 1 Variation of 1-MHz dielectric constant in $(\text{Ta}_2\text{O}_5)_{1-x}(\text{TiO}_2)_x$ polycrystalline ceramics at 20 °C as a function of composition.

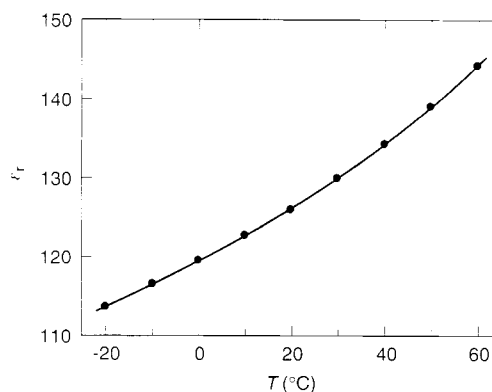


FIG. 2 Variation of the 1-MHz dielectric constant of a 0.92:0.08 Ta_2O_5 : TiO_2 polycrystalline ceramic between –20 and +60 °C.

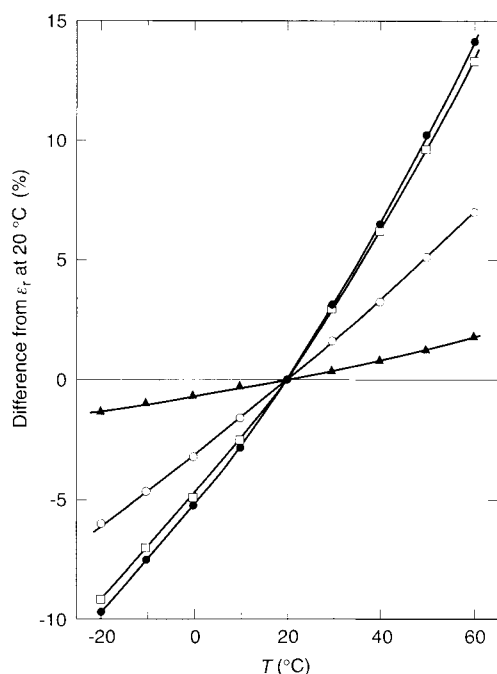


FIG. 3 Comparison of the relative variation of the 1-MHz dielectric constant with temperature at $\sim 20^\circ\text{C}$ for Ta_2O_5 and various Ta_2O_5 - TiO_2 compositions. Filled triangles, Ta_2O_5 , $\epsilon_{r,20} = 36$; open circles, 0.85:0.15 Ta_2O_5 : TiO_2 , $\epsilon_{r,20} = 69$; open squares, 0.94:0.06 Ta_2O_5 : TiO_2 , $\epsilon_{r,20} = 94$; filled circles, 0.92:0.08 Ta_2O_5 : TiO_2 , $\epsilon_{r,20} = 126$.

frequencies is due to measurement circuit imbalance and is not intrinsic to the material.

Characterization of the $(\text{Ta}_2\text{O}_5)_{1-x}(\text{TiO}_2)_x$ materials by conventional powder X-ray diffraction showed that the enhanced dielectric constant for 8–15% TiO_2 content is associated with the appearance of the H' monoclinic Ta_2O_5 solid-solution phase, in agreement with the published phase-equilibria diagram⁷. The crystal structure of Ta_2O_5 itself is complex, involving highly distorted TaO_7 and TaO_6 coordination polyhedra, in a structure whose periodicity is known to be sensitive to small quantities of additives⁸. Our preliminary experiments with a variety of additives known to modulate the structure in a manner similar to that for TiO_2 substitution showed that enhanced dielectric con-

stant was not generally observed, suggesting that the special case of Ti accommodation in the distorted coordination geometries afforded by the Ta_2O_5 structural framework is what causes the enhancement of the dielectric constant in the present case.

In conclusion, we have shown that modification of Ta_2O_5 through substitution with small quantities of TiO_2 greatly enhances the dielectric constant. Microelectronic devices employing the new material in place of pure Ta_2O_5 would be expected to boast a threefold area reduction for their capacitive components, perhaps even eliminating the need for complex three-dimensional capacitor geometries often currently employed to yield acceptable capacitances in small-area components. Because Ta and Ti are sometimes employed in present silicon-processing procedures, it may be possible to incorporate the new material into present device-processing techniques without greatly perturbing the manufacturing process. The stability of 0.92:0.08 Ta_2O_5 : TiO_2 to high temperatures suggests that it would be robust to fabrication procedures for microelectronic components. The study of the processing and characteristics of the new material in thin-film form should be of significant interest. □

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Trends in high-frequency climate variability in the twentieth century

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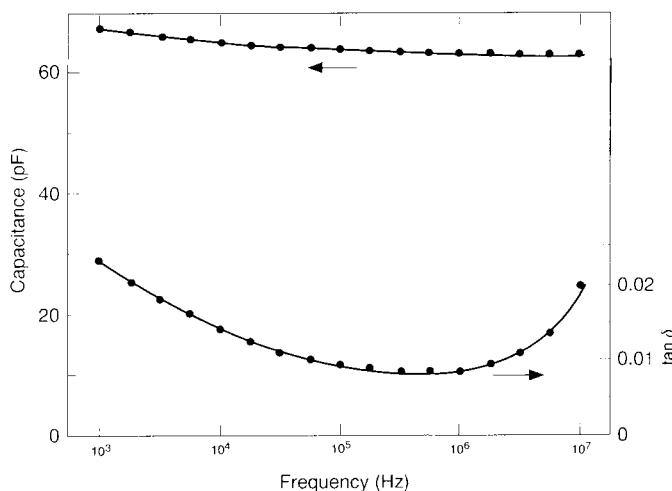


FIG. 4 Frequency dependence of the capacitance (dielectric constant at 1 MHz = 130) and $\tan \delta$ of a polycrystalline pellet of 0.92:0.08 Ta_2O_5 : TiO_2 at 30°C (bias level, 0 V; oscillation voltage, 1.0).

HIGH-FREQUENCY climate variability is a fundamental aspect of climate. Understanding climate change demands attention to changes in climate variability and extremes¹, but knowledge of the recent behaviour of these variables has been limited by the unavailability of long-term high-resolution data. Climate simulations incorporating increased greenhouse-gas concentrations^{2–9} indicate that a warmer climate could result in a decrease in high-frequency temperature variability (analogous to the decrease in variability observed from the poles to the tropics, and from winter to summer¹⁰) and an increase in the proportion of precipitation occurring in extreme events. Here we analyse high-frequency temperature and precipitation data from hundreds of sites spread over Australia, China, the former Soviet Union and the United States over the past 30 to 80 years. Day-to-day temperature variability is seen to have decreased in the Northern Hemisphere, and—at least within the United States—the proportion of total precipitation contributed by extreme, one-day events has increased significantly. We find that although the notion of a recent increase in interannual temperature variability is supported by data from the past few decades¹¹, the longer data records indicate that this trend is an aberration.

Changes in interannual and intraseasonal temperature variability and the frequency distribution of precipitation were calculated within several countries. Stations used included: a network of 187 well-distributed stations of the highest quality predominantly from the USA Historical Climatology Network (HCN)¹², 223 stations across the former Soviet Union (FSU)¹³, 197 stations in China¹⁴, and 40 reference climate stations in Australia mostly derived from the work of Plummer *et al.*¹⁵. The period of record used for temperature (precipitation) varied by country: USA, 1911–1992 (1911–1994); FSU 1935–1989 (1967–1989); China, 1952–1989 (1952–1989); and Australia, 1961–1993 (no data). During these periods, at least 85% of the stations reported each year, except in the FSU during past three years, where only 50% of the stations were available for analysis.

There are many different ways to define temperature variability, and results will clearly be dependent on the statistic chosen, for example the difference in the daily high and low temperatures (the magnitude of the diurnal temperature range), the difference of the mean temperature from one day to the next, and the standard deviation of temperature. The variability of temperature is also frequency dependent, for example interannual versus intraseasonal measures of variability. All measures of variability depend on a difference from some reference point, such as a moving average, a long-term mean, or the previous discrete value. In this analysis, temperature variability was defined as the mean of a series of values defined by the absolute value of the difference in temperature between two adjacent discrete time periods. Such a definition is less prone to confounding the influence of high- and low-frequency variability than are more commonly used measures of variability, such as the standard deviation. The standard deviation is insensitive to the temporal position of large positive or negative departures from the mean. It can inadvertently reflect changes due to a change in the mean (for example, the time series 0,0,0,0,1,1,1,1 and 0,1,0,1,0,1,0,1 have identical standard deviations) or a shift in the annual cycle¹⁶. Similarly, there are disadvantages of using running averages and other filters as a reference to calculate variability, as these weighting functions do not break cleanly at discrete time intervals, making interpretation more difficult because the basic temperature observations are discrete, not continuous.

The daily maxima, minima and average temperatures and the magnitude of the diurnal temperature ranges were used to estimate decadal changes of temperature variability. For each variable, daily anomalies were calculated using the first three harmonics of the period-of-record mean annual daily temperature time series as the reference. Little, if any, additional variance was explained with high-order harmonics. Several time intervals of temperature differences were defined to determine trends of intraseasonal variability (1-day, 2-day, 5-day, 10-day and 30-day) and interannual variability (12-month). Temperature variability was calculated from the absolute value of the difference of the mean temperature anomaly from time period i to $i+1$. Intraseasonal differences relative to the 1-day to 30-day time

intervals were calculated by using partly overlapping running differences, for example for 1-day differences day 2 minus day 1, day 3 minus day 2, and so on, and for 30-day differences days 31 to 60 minus days 1 to 30, days 32 to 61 minus days 2 to 31, days 33 to 62 minus days 3 to 32, and so on. Strictly non-overlapping differences artificially constrain the number of differences calculated within each season, for example only two 30-day differences per season, and leads to noisy seasonal estimates. For limited samples the partly overlapping differences can be shown to be more efficient estimators in time-series simulation with known spectral density changes. Moreover, the choice of non-overlapping or partly overlapping differences has no effect on aliasing low-frequency changes of interest in our analysis because no smoothing occurs between years before statistical tests of significance. Changes of interannual variability calculated from averages of the four seasons, where differences in seasonal computations do not span seasons, for example the last summer month relative to the first autumn month, will not be equivalent to changes calculated by integrating through the year as calculated here. Subsequent to calculating differences for each station, the values were arithmetically averaged within 9 to 23 regions per country (depending on size) and then area-averaged across the countries.

Results reveal that intraseasonal temperature variability has generally decreased in the Northern Hemisphere (Fig. 1), but with mixed trends over Australia. Significant decreases (Table 1) occur for all elements in the USA at the shortest time intervals (day-to-day), and for the diurnal range within the FSU and China. The decreases in intraseasonal variability in the USA arise primarily from decreases during spring and summer, and in China the decreases are especially pronounced during spring (not shown). Statistically significant decreases in the FSU are also observed during spring and summer for short-time-averaged differences (through 5-day time averages), but in Australia no significant decreases were apparent. Of particular interest, no significant increases (Fig. 1), regardless of the test, are evident in any country (although there is a tendency for increased interannual variability over the USA, Australia and the FSU during the past few decades). Moreover, of the 96 annual trends depicted in Table 1, only 18 are positive, and 11 of these are observed over Australia where the average intraseasonal variability is actually stronger in the warm season, in contrast with the other regions.

Daily precipitation totals were aggregated into five categories of precipitation ranging from very light (≤ 2.54 mm d^{-1}) to extreme (> 50.8 mm d^{-1}). The proportion of seasonal and annual precipitation falling in each of these categories was calculated for every station each year for each region within the USA, FSU and China. In a similar way to temperature, area averages were calculated for each national mean.

The impact of time-dependent missing daily data can bias precipitation trends if assumed to be zero or at the daily average for the month. To prevent biases, the location, scale and shape parameters of the gamma distribution were calculated, based on

FIG. 1. Variations and trends of the difference in average temperature for various averaging intervals. Statistically significant (see Table 1) trends (0.01 significance level) are depicted in bold, as calculated from test (C) described in Table 1, along with the variations of the annual values after smoothing with a nine-point binomial filter.

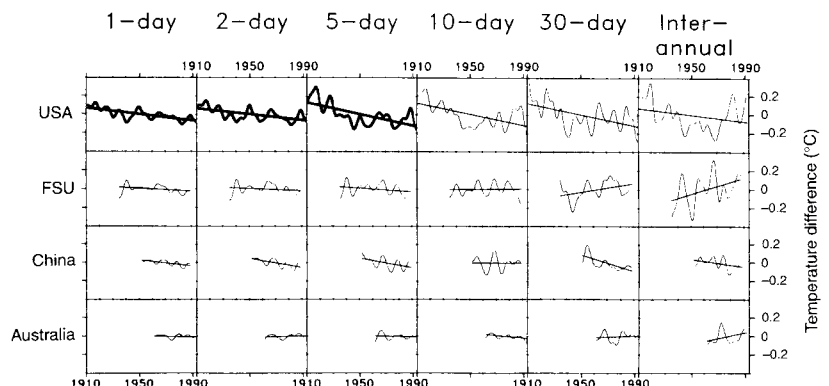


TABLE 1 Sign of the trend for various measures of daily temperature variability and their associated statistical significance

Element	Australia (1962–1993)	China (1952–1989)	FSU (1935–1989)	USA (1911–1994)
1-day				
Maximum	+	–	–	–†
Average	–	–	–	–†
Minimum	–	–	–	–†
Range	+	–	–†	–†
2-day				
Maximum	+	–	–	–†
Average	+	–	–	–†
Minimum	+	–	–	–
Range	+	–†	–†	–†
5-day				
Maximum	+	–	–	–†
Average	–	–	–	–†
Minimum	–	–	–	–†
Range	–	–†	–†	–†
10-day				
Maximum	–	–	+	–†
Average	–	–	–	–*
Minimum	–†	–	–	–
Range	–	–†	–†	–†
30-day				
Maximum	+	–	+	–
Average	+	–	+	–
Minimum	–	–†	+	–
Range	–	–	–†	–
Interannual				
Maximum	+	–	+	–
Average	+	–	+	–
Minimum	–	–	+	–
Range	–	–	–	–†

Statistical significance (0.01 probability level) is indicated based on three conditionally dependent tests in the following order: (a) a t-test of the slope (*), (b), a Monte Carlo simulation based of a first-order autoregressive model (†), and (c), a Monte Carlo simulation of the best fit autoregressive moving-average model²⁴ up to order 4 (‡). The second and third tests are based on Monte Carlo simulations of the best-fit autoregressive model to the observed data. The Monte Carlo simulation uses the model fit to generate 1,000 time series of length equal to the observed record. The percentage of time that the absolute value of the simulated series exceeded the observed trend is a direct measure of the statistical significance. The most appropriate model is selected on the basis of the Bayesian information criterion²⁵. The trend was removed before fitting each ARMA model. Range is the magnitude of the diurnal temperature range.

the distribution of daily precipitation for months with complete data. The gamma distribution was then used to estimate missing daily precipitation. This distribution has been found to realistically represent precipitation processes^{17–19}. Roughly 15% of all months had some missing data. Before estimating the amount of daily precipitation a random-number generator was used to determine whether precipitation occurred, by using the empirical probability of the observed frequency of precipitation within the month. Other possible heterogeneities that can affect our analysis include changing observing times that alias the diurnal cycle of precipitation. We constrained our stations such that there was no systematic change in observing time, and we have tested subsets of the networks with constant observing times with no change in results. Other biases include those affecting precipitation measurements in the FSU²⁰. Monthly adjustments given by Groisman *et al.*²⁰ require more work before applying them to daily data. As a result, we confine our analysis over the FSU to the period since 1967, when observing methods became more stable.

Our analysis is sensitive to shifts in the distribution of precipitation, even when there is no overall change in the total precipitation; it is non-parametric and not constrained by a specific distribution (except for estimates of missing data); and changes in each category can be directly related to specific precipitation amounts. Moreover, if the only change in the distribution is a change in mean, but the shape and scale of the distribution

remain unchanged, no change will be reflected in the proportion of precipitation within any category.

Over the USA (Fig. 2) a clear signal emerges: there is an increase in the proportion of precipitation derived from the extreme precipitation category ($>50.8 \text{ mm d}^{-1}$) whereas the proportion of precipitation derived from the moderate ($12.7\text{--}25.4 \text{ mm d}^{-1}$) and light precipitation categories ($2.5\text{--}12.7 \text{ mm d}^{-1}$) decreases. The increase in the extreme category in the USA is a result of increases during all seasons, but is predominant during the convective seasons, especially summer (June–August) with spring (March–May) also contributing. The trend of total precipitation during spring and summer in the USA has been near zero²¹. Within the USA, all areas of the country participate in the increase except the far west and the southeast. Since 1911, the increase (decrease) in the proportion of precipitation derived from extreme (light and moderate) precipitation rates has exceeded 2% of the total summer precipitation, and this translates to an average of about one additional extreme precipitation event every two years. It is noteworthy that no change in precipitation intensity can be detected if precipitation intensity is defined as the total precipitation divided by the number of days with precipitation. The two quantities, total seasonal or annual precipitation and the number of days with precipitation, are highly correlated (~ 0.9).

Stations selected in the USA were predominantly rural stations in the USA HCN, thereby circumventing the possibility of urban-induced increases in precipitation²². Another potential bias that has been estimated to occur at about 5% (Data Operations Branch, National Climatic Data Center) of the stations relates to observations derived from businesses that close during the weekend and report a 3-day total precipitation on Monday morning as opposed to the expected 1-day total. Although there is some indirect evidence that there are a greater number of stations now closing during weekends, the proportion of stations with such a practice in the US HCN is not significant, as changes in the proportion of precipitation falling in each category over 3 days were very similar to the 1-day totals.

In agreement with model projections of a warmer world, the evidence suggests a Northern Hemisphere decrease in short-term temperature variability with some very significant decreases evident in the USA consistent with several model projections^{6,7}. The observations also show that the proportion of total precipitation derived from extreme precipitation events reflects large increases

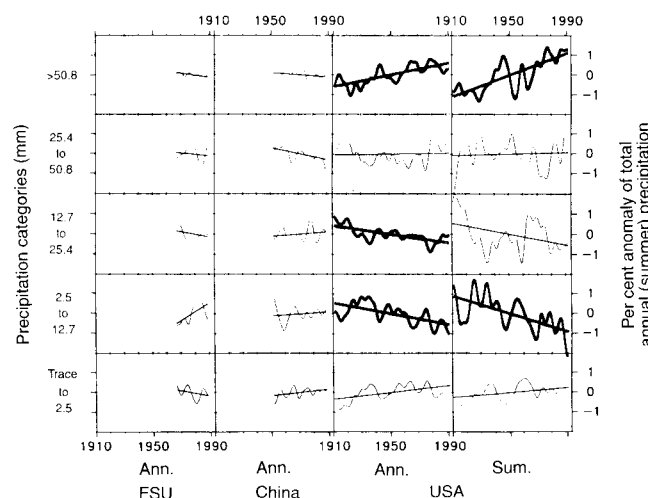


FIG. 2 Variations and trends in the percentage of total precipitation (given on the right ordinate) in each of five separate precipitation categories (identified on the left ordinate). Statistically significant trends (bold) are calculated as in Fig. 1. ('Ann.' represents annual averages and 'Sum.' represents the summer averages (June, July and August); other abbreviations are given in Fig. 1 legend.

in such events during the warm season over the United States. In contrast, there is little systematic change in precipitation variability over the FSU or China, even considering regional changes within these countries, although the substantially shorter records may mask trends. We note that a recent analysis of northeastern Australia warm-season precipitation²³ also supports an increase in the intensity of heavy precipitation during the twentieth century. □

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Microbial mediation as a possible mechanism for natural dolomite formation at low temperatures

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DOLOMITE ($\text{CaMg}(\text{CO}_3)_2$) is a common carbonate mineral which is found in much greater abundance in ancient rocks than in modern carbonate environments. Why this is so remains a mystery. Over the past 30 years, dolomite formation has been observed in several modern environments, and various thermodynamic, kinetic and hydrological factors have been proposed to explain its formation^{1,2}. But attempts to precipitate dolomite at low temperatures in the laboratory have been unsuccessful^{3,4}, and the 'dolomite problem' remains a source of controversy in sedimentary geology^{5–7}. Here we describe experiments in which a ferroan dolomite with a fairly high degree of cation order was precipitated in the presence of sulphate-reducing bacteria from the *Desulfovibrio* group. We propose that the direct mediation of these anaerobes can overcome the kinetic barrier to dolomite nucleation, and that they may play an active role in the formation of this mineral in natural environments.

The recent discovery⁸ of primary dolomite precipitation in Lagoa Vermelha, a small (1.9 km²), shallow-water (<2 m), isolated coastal lagoon situated between Rio de Janeiro and Cabo Frio, Brazil (22° 56' S, 42° 23' W) adds another dimension—direct microbial mediation—to our understanding of dolomite formation. Within an anoxic black sludge layer at the base of the water column, poorly ordered Ca-dolomite and high-Mg-calcite precipitate in association with sulphate-reducing bacteria from the *Desulfovibrio* group. Early diagenesis of the authigenic carbonates begins within the upper 15 cm of the sediments, with sulphate-reducing bacteria promoting continued dolomite precipitation with a reordering of the crystal structure and the

TABLE 1 Electron diffraction analysis of experimental carbonate

Miller index (hkl)	Sample <i>d</i> (hkl) Å	Dolomite <i>d</i> (hkl) Å	Ankerite <i>d</i> (hkl) Å	Calcite <i>d</i> (hkl) Å
102	3.71	3.69	3.70	3.86
110	2.40	2.41	2.41	2.49
122	1.55	1.54	1.55	1.60
104	2.94	2.89	2.90	3.03

Standard parameters are from ASTM, refs 12–88.

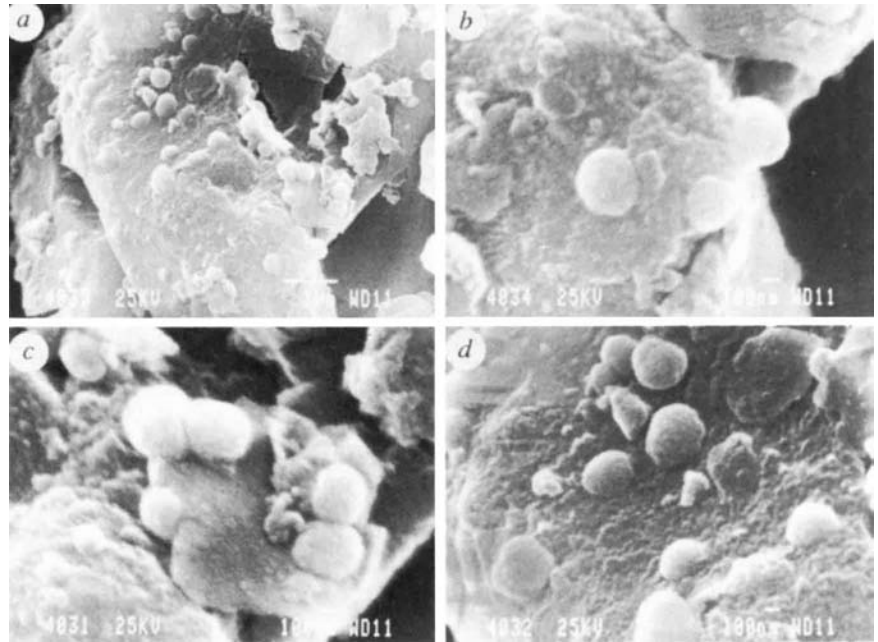
formation of dolomite concretions (C.V. and J.A.McK., manuscript in preparation). Evidence for microbial mediation can be observed in secondary electron images in the scanning electron microscope (SEM), which show subspherical nanobacteria embedded in the surface of the dolomite. Folk^{9,10} has noted the association of dolomite and nanobacteria in modern and ancient samples and suggested that the nanobacteria might be the active agent of dolomite precipitation.

As the black sludge in Lagoa Vermelha apparently contains an active microbial community that is possibly capable of mediating dolomite precipitation, we did a bacterial culture experiment with samples from this layer to simulate in the laboratory the dolomite-producing process observed in the lagoon. The experiment was done at the Idaho National Engineering Laboratory, USA. A Postgate B medium (number 53; ref. 11), modified to match the chemical composition of the Lagoa Vermelha waters, was used for the experiment. In the first step, a sample of sludge was added to the growth medium and kept under anoxic conditions for one week, after which the presence of sulphate-reducing bacteria was confirmed by FeS precipitation. One ml of the positive supernatant was then added to 20-ml crimped vials containing the growth medium and quartz sand substrate, which had been previously washed with HCl solution to eliminate any carbonate. Again, confirmation of the presence of sulphate-reducing bacteria was indicated by FeS precipitation after one week. Positive samples were incubated in a refrigerator (~4 °C), for more than one year. The tubes were transported to ETH, Zürich, where they were opened, with a notable release of H₂S. The cultures were filtered on 0.2 µm polycarbonate filters. Aggregates of the quartz sand, weakly cemented by a carbonate which effervesces when a 10% HCl solution is applied, were concentrated under a binocular microscope. A sterile control sample was prepared under the same conditions, but showed no evidence of sulphate-reducing bacteria or carbonate precipitation after one year.

Viewed in an SEM secondary electron image (Fig. 1a), the surface of the quartz substrate is covered with a knobby coating

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FIG. 1 SEM secondary electron images showing: a, an overview of the surface of the quartz substrate used in the bacterial cultural experiment—note that the surface is partially covered with a knobby carbonate coating that appears to be colonized by subspherical nanobacteria (scale bar, 1 μm); b, close-up of a section of the carbonate coating that was analysed for elemental identification using the EDX attachment (see Fig. 2; scale bar, 0.1 μm); c, close-up of twinned nanobacteria that may have been imaged in the process of reproduction via cell division (binary fission) (scale bar, 0.1 μm); d, close-up of the carbonate coating, illustrating the apparent stages in the process of its bacterial formation from detached subspherical nanobacteria encrusted by nanocrystals of carbonate to attached or embedded bodies that become rounded bumps entombed in the coating, which in turn merge to cover the surface of the substrate (scale bar, 0.1 μm). SEM images were produced by a JEOL JSM 840 SEM coupled with an energy dispersive X-ray spectrometer (TRACOR N 2000) for elemental identification.



that appears to be colonized by subspherical nanobacteria. Figure 2 is an energy-dispersive X-ray spectrum centred on the colonized coating of the quartz grain shown in Fig. 1b. The spectrum contains high-intensity peaks for Mg and Ca, as well as lesser peaks for Fe, indicating that the coating would be consistent with a Ca, Mg, Fe carbonate. X-ray diffraction analysis of the aggregates shows that, apart from the quartz substrate, they comprise predominantly rhombohedral carbonate (Fig. 3). The sharply defined major peak, the $d(104)$ reflection, occurs at 2.90 $\text{\AA}$ ($30.79^\circ 2\theta$) in the diffraction pattern, indicating the presence of dolomite. This position is shifted slightly from the major peak for ideal dolomite, which occurs at 2.89 $\text{\AA}$ ($30.99^\circ 2\theta$). The X-ray diffraction pattern for the experimental dolomite shows secondary peaks (Fig. 3). In particular, the presence of the 015 peak is consistent with its being an ordered dolomite¹².

Electron diffraction analysis of the aggregates using transmission electron microscopy (TEM) was done to determine more exactly the crystallographic characteristics of the experimental carbonate. The $d(hkl)$ parameters (in $\text{\AA}$) for the carbonate,

measured from the TEM negative and corrected by a factor related to the TEM characteristics, are given in Table 1, together with the $d(hkl)$ values for standard dolomite, ankerite and calcite. A comparison of the $d(hkl)$ values indicates that the experimental carbonate has a composition lying between dolomite and ankerite—in other words, a ferroan dolomite. The identification of ferroan dolomite for the carbonate mineral that weakly binds the aggregates of the quartz substrate used in the bacterial culture experiment is consistent with the qualitative elemental scan (Fig. 2). The presence of Fe in the dolomite structure is not inconsistent with the activity of sulphate-reducing bacteria under anoxic conditions. Also, Fe is a component of the culture medium. Together, our analyses provide conclusive evidence that the bacterial production of dolomite can be achieved in low-temperature anoxic conditions in a relatively short-time.

The bacterial bodies have different sizes, with an average diameter of 0.2 μm (Fig. 1b–d). At the higher magnification, the nanobacteria appear to be encrusted by nanocrystals of dolomite, which apparently precipitates on the outer surface of the

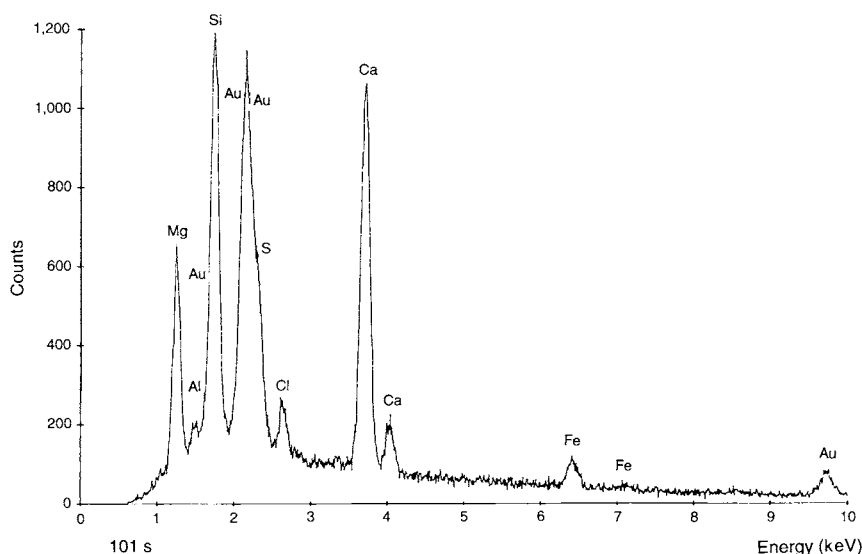


FIG. 2 An energy-dispersive X-ray (EDX) spectrum of the surface coating on the quartz grain shown in Fig. 1b. Note the intensity of the Mg, Ca and Fe peaks, the dominant elements identified in the sample (apart from Si for the quartz substrate), and Au from the gold coating used to achieve a better resolution of the SEM image. The relative peak height of Mg is less than Ca because of the attenuation of Mg X-rays in the EDX system.

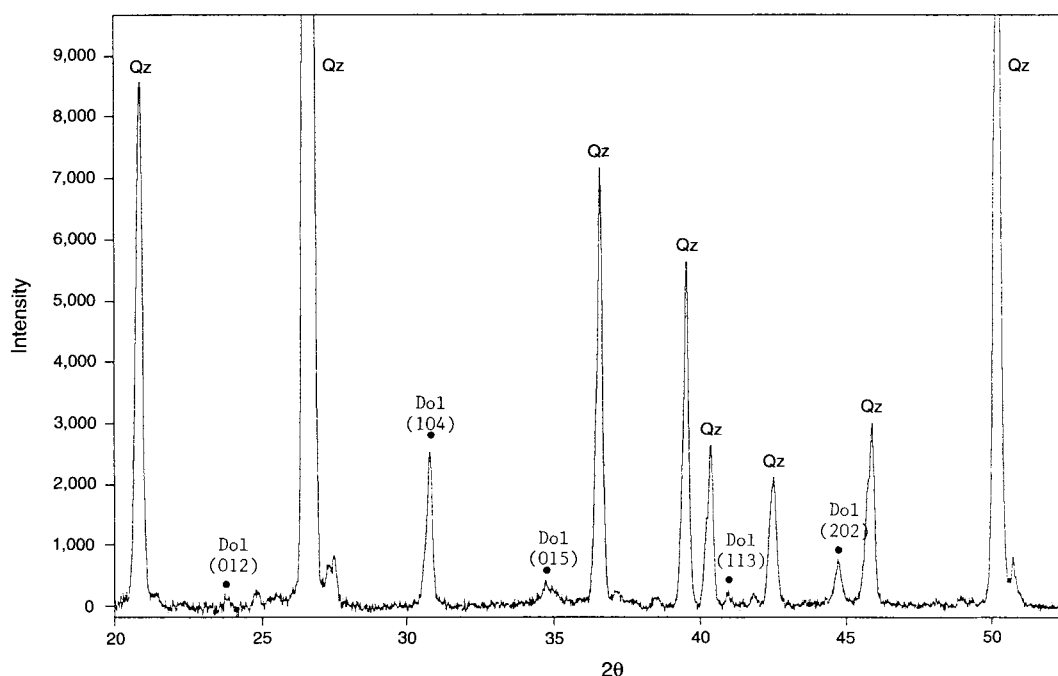


FIG. 3 X-ray diffractogram (20° to 55° 2θ) of the bulk powdered sample recovered from the bacterial experiment. The XRD analysis was produced on a STOE STADIP transmission goniometer. The peaks in the

powder diffraction pattern clearly correspond to quartz plus a rhombohedral carbonate. The presence of the 'ordering reflection' 015 peak indicates that the mineral is a fully ordered dolomite¹².

bacterial bodies. Some of the nanobacteria may be in the process of reproduction by cell division (Fig. 1c). Figure 1d shows that, as the process of bacterial precipitation proceeds, the bodies no longer occur as discrete subspheres on the surface but become attached or embedded in it. Finally, they become rounded bumps entombed in the coating, which in turn covers the surface of the substrate. As the nanobacteria use the quartz surface as a growth substrate, the precipitation of dolomite in conjunction with the microbial activity apparently cements the quartz grains to form the observed aggregates.

The $\delta^{18}\text{O}_{\text{PDB}}$ and $\delta^{13}\text{C}_{\text{PDB}}$ values of the ferroan dolomite are -6.7 and -12.8% , respectively. These values are further evidence that the dolomite is not a contaminant but a product of the bacterial culture experiment. As the $\delta^{18}\text{O}_{\text{SMOW}}$ value of the water used in the experiment was -14.0% , the dolomite appears isotopically to mirror the solution from which it precipitated. The negative $\delta^{13}\text{C}_{\text{PDB}}$ value of the dolomite reflects an input of organic carbon released as a product of sulphate reduction. Because it has not been previously possible to produce dolomite in the laboratory at Earth-surface conditions, calibration of its isotope fractionation, as well as minor cation distributions in dolomite, could not be achieved. We suggest that future bacterial culture experiments will furnish us with a valuable method to

make these calibrations, which are needed to interpret conditions of ancient dolomite formation.

The results of this bacterial culture experiment provide strong evidence for the involvement of bacteria (possibly including sulphate-reducing bacteria) in the precipitation of dolomite. In 1928, Nadson reported results from a similar bacterial culture experiment in which he used sulphate-reducing bacteria from anoxic surface sediments in a Russian salt lake to produce very small amounts of an authigenic carbonate¹³. A qualitative chemical analysis of the carbonate led him to propose that dolomite might have been precipitated in association with the anaerobes; he suggested that 'understanding the essential role by this bacterial phenomenon may be the solution to the dolomite problem'.

As microbial activity apparently governs dolomite precipitation in Lagoa Vermelha, we propose that microorganisms may be important as dolomite nucleation centres in this environment, as well as in other environments of dolomite formation such as coastal sabkhas and carbonate platforms. Considering the unequal distribution of dolomite in the geological record, we further suggest that the bacterial production of dolomite was far more significant in the past than at present, in particular in the Proterozoic when the dolomite/limestone ratio was about 3:1 (ref. 14). □

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Hot subterranean biosphere in a continental oil reservoir

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THE presence of high concentrations of hyperthermophilic archaea in Alaskan oil fields has been attributed to viable hyperthermophiles in low concentrations in the injected sea water, but the existence of an indigenous community within the reservoir was ruled out¹. Here we present evidence for the existence of indigenous thermophilic bacteria and hyperthermophilic archaea from a continental petroleum reservoir about 1,670 m below the surface. The thermophilic isolates were repeatedly obtained from different wells and thrived in media similar to conditions in the wells, suggesting that these isolates are members of a deep indigenous thermophilic community. The unexpected presence of marine hyperthermophilic archaea in a deep continental environment extends the known ecological habitat of this group of organisms, and their unusual co-existence with terrestrial bacteria suggests that thermophiles may be widespread deep in the crust of the earth.

To detect the possible existence of thermophilic microorganisms in a deep subterranean biotope, well-head samples were collected from four wells (A, B, C and D) located in the East Paris Basin on a large oil reservoir about 1,670 m below the surface (200 m above sea level). Since 1988, most of the oil fields have been flooded by fresh water from an aquifer about 800 m below the surface to enhance oil recovery. However, the wells sampled in August 1993 have never been flooded. The measured concentrations of chloride (74–171 mM) and sulphate (5.1–7.8 mM) of the collected water phases were low compared with those of sea water (~540 mM and 28 mM, respectively). The *in situ* temperature of the water ranged between 65 and 70 °C, and at the sampling points the emergent production fluids had cooled to 50 °C. The *in situ* pressure was between 50 and 160 atm.

The existence of thermophiles within the production fluids was investigated by inoculating sterile, anaerobic culture media with the water phases of the samples (Table 1). Most of the culture media became turbid when incubated for 18–72 h at 65 °C and 80 °C. Cultures growing at 65 °C in the presence of cystine showed mixtures of regular cocci, abundant sheathed rods ('*Thermotoga*-like' bacteria²) and thin, long sporulating rods. At 80 °C, the cultures were predominantly regular paired cocci. H₂S was formed as a result of cystine reduction. Cultures enriched at 65 °C in the absence of cystine produced tiny straight rods that did not form spores, and at 80 °C the enrichments were dominated by irregular coccoid cells showing a bluish-green fluorescence at 420 nm ('*Archaeoglobus*-like' sulphate reducers³). H₂S was also produced in these enrichments.

Pure cultures were successfully obtained through purification of one colony of each morphological and/or metabolic type (Table 2). DNA from isolates SL2, SL4 and SL5 hybridized strongly (>70%)⁴ with DNA from their corresponding bacterial or archaeal relatives^{5–7} (Table 2). Based on DNA–DNA hybridization, the isolate SL1 did not show species-level similarity with other members of the 'Thermotogales', and from 16S ribosomal RNA analysis it was recognized as a new species ('*T. subterranea*')⁸. Isolate SL3 is identical to *Thermoanaerobacter*

TABLE 1 Thermophiles and hyperthermophiles from the four oil-field waters

Sample (well)	Enrichment cultures with cystine at		Enrichment cultures without cystine at			
	65 °C	80 °C	65 °C		80 °C	
			L+A	B+P	L+A	B+P
A	+	+	+	+	+	–
B	+	+	+	–	+	–
C	+	+	+	+	+	–
D	+	+	+	–	+	–

Samples of oil–water phases were taken from the production well-heads through a connection on the production line and collected in sterile 250-ml glass bottles. Hungate tubes containing 9 ml of different cultivation media^{12,20} were inoculated with the samples (1 ml) and incubated for 3 days without shaking. The tubes were pressurized (1 atm) with N₂ (100%). NaCl concentration of the enrichment media was adjusted to 10 g l⁻¹. Media without cystine were supplemented with 10 mM lactate (L) and 15 mM acetate (A) or 2 mM benzoate (B) and 10 mM propionate (P) as electron donors for sulphate reduction. Our attempts to enrich for methanogens and aerobic heterotrophic thermophiles failed. Symbols: +, positive; –, no growth.

thermohydrosulfuricus, based on the G + C mol% (35%) (ref. 9), cellular, physiological and metabolic characteristics¹⁰.

At the *in situ* temperature (70 °C), growth of the reservoir isolates was unaffected by 50 and 160 atm, the *in situ* minimum and maximum pressures. On cultivation on the oil–water phase, the initial cell concentration of both sulphate reducers, *T. commune*-SL4 and *A. fulgidus*-SL5, increased four- to fivefold (Fig. 1). The *in situ* bioconversions of the crude oil fractions (probably aliphatic and aromatic hydrocarbons¹¹) and sulphate reduction may provide carbon sources, electron donors and acceptors that could promote the growth of the other sulphidogens inhabiting the reservoir. The newly isolated strains of the genera *Thermodesulfobacterium* and *Thermoanaerobacter* have optimal physiological characteristics identical to those displayed by the previously described type strains^{6,10}, and do not exhibit a pronounced salt requirement or a significant salt tolerance^{12,13}. Similarly, the *Archaeoglobus* and *Thermococcus* isolates share physiological characteristics with previously described strains^{5,7} and have an obligate salt requirement which indicates marine

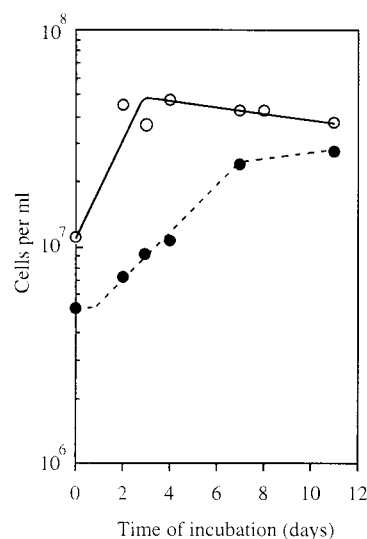


FIG. 1 Growth of *T. commune*-SL4 (open circles) and *A. fulgidus*-SL5 (filled circles) cultivated anaerobically on sterilized oil–water phase from well B at 70 °C under atmospheric pressure. Bacterial growth was monitored by direct staining with acridine orange and counting by epifluorescence microscopy²⁴.

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TABLE 2 Temperature growth range and NaCl concentrations for growth of the isolates from the Paris Basin oil reservoir

Isolated (sample)	Electron acceptors	Growth temperature (°C)			NaCl concentration for growth (g l ⁻¹)			Species
		min.	opt.	max.	min.	opt.	max.	
SL1 (B)	S ₂ O ₃ ²⁻ , cystine	50	70	80	0	12	24	<i>Thermotoga subterranea</i> sp. nov.*
SL2 (B)	S ⁰ , cystine	50	85	98	3	24–30	65	<i>Thermococcus litoralis</i>
SL3 (C)	SO ₃ ²⁻ , S ₂ O ₃ ²⁻	40	70	75	0	0	30	<i>Thermoanaerobacter thermohydrosulfuricus</i>
SL4 (B)	SO ₄ ²⁻	60	70	82	0	0	20	<i>Thermodesulfobacterium commune</i>
SL5 (C)	SO ₄ ²⁻	64	80	88	0	20–24	36	<i>Archaeoglobus fulgidus</i>

One colony of each isolate was obtained from shake tubes solidified by 0.7–1% Gelrite²¹ (Kelco, San Diego) incubated at 65 °C (isolates SL1, SL3 and SL4) and 75 °C (isolates SL2 and SL5). Temperature growth range and NaCl concentrations for growth were determined in appropriate media^{12,20,22}. To determine the optimal NaCl concentrations for growth, the NaCl concentrations were varied while maintaining the concentrations of the other inorganic components. For identification, genomic DNAs of isolates and their possible relatives were purified by CsCl gradient centrifugation²⁰. Genetic relatedness was investigated by slot-blot DNA/DNA hybridization (Bio-Dot apparatus, BioRad, Richmond, CA) using ECL random prime system (Amersham, Les Ulis, France). Increasing amounts of target DNA (25–100 ng) denatured in 0.4 M NaOH were slotted onto a hybridization membrane (Zeta-Probe, Bio-Rad) and probed with 200 ng of labelled tracer DNA²³. For each duplicate of DNA/DNA association (15 h in buffer 4 × SSC with formamide, 0.5% blocking agent, 5% dextran sulphate, 100 µl ml⁻¹ denatured sheared salmon sperm DNA), the temperature of hybridization chosen was optimal²⁰. Final high stringent washes were performed at 65 °C in 0.5 × SSC, 0.1% SDS and chemoluminescent detection was performed according to the manufacturer's instructions. Hybridization signals were recorded by autoradiography (Hyperfilm-ECL) and scanned with a densitometer²³. Signal (maximum peak area) produced by self-hybridization of the probe with homologous target DNA was set as 100%. DNA of the following strains were used: *Thermococcus celer* (DSM 2476), *T. stetteri* (DSM 5262), *T. litoralis* (DSM 5474), *Thermotoga maritima* (DSM 3109), *T. neapolitana* (DSM 4359), *T. thermarum* (DSM 5069), *Fervidobacterium nodosum* (DSM 5306), *F. islandicum* (DSM 5733), *Thermosipho africanus* (DSM 5309), *Thermodesulfobacterium commune* (DSM 2178), *T. mobile* (DSM 1276), *Archaeoglobus fulgidus* (DSM 4304), and *A. profundus* (DSM 5631).

* Poor and slow growth.

origins even though they were obtained from a continental reservoir.

The origin of thermophilic microorganisms in oil reservoirs has been attributed to either the occurrence of indigenous inhabitants¹⁴ or the introduction and proliferation of exogenous contaminants transported with the injection water¹ or during drilling¹⁵. The strictly anaerobic mode of life and the minimum growth temperatures (Table 2) displayed by the reservoir thermophiles suggest that they are not active in aerated conditions or at ambient temperatures¹. The introduction of bacteria by atmospheric transport or their growth in the formation water before reinjection for secondary recovery, thus inoculating the reservoir in a manner similar to that described previously¹, would be highly unlikely. As the minimum growth temperatures of the isolates are below the temperature recorded for field waters (65–70 °C), the possibility that the samples were contaminated by bacteria growing in the production well-head or other cooler places of the production system cannot be completely ruled out. However, as the same morphological and metabolic types were recovered repeatedly from the production fluids of four separate well-heads (Table 1), and both the archaeal and bacterial isolates from these well-heads grow in the same temperature range as that measured in the reservoirs from which they originated, these thermophiles are probably representatives of the autochthonous microflora.

These findings are in agreement with recent demonstrations of other deep subsurface environments such as aquifers¹⁶, groundwater from a borehole in granitic rock¹⁷, and marine sediments¹⁸. The occurrence of these thermophiles in this deep location suggests that they may have been deposited with the original sediment and survived over geological time^{14,19}. The extent to which the geological and hydrological conditions influenced the survival, diversity and evolution of the present community is unclear. □

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A new giant carnivorous dinosaur from the Cretaceous of Patagonia

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LARGE carnivorous animals, the top members of the trophic chain, are rare, and flesh-eating dinosaurs were rarer still. For years the only known giant theropods were *Tyrannosaurus rex*¹ and the poorly known *Deinocoelurus mirificus*², both from the Northern Hemisphere, but many important new dinosaurs have been discovered in the Southern Hemisphere during the past decade, considerably increasing our knowledge of ancient ecosystems. Here we report a new giant carnivorous dinosaur from the Upper Cretaceous of northwestern Patagonia (Argentina). This new taxon, *Giganotosaurus carolinii* gen. et. sp. nov., is characterized by a

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proportionally low skull, a reduced shoulder girdle, and robust vertebrae and hind limbs. It represents a primitive evolutionary iteration of large theropods, and provides an opportunity to examine the Gondwanan dinosaur palaeocommunities and their relationships to those from Laurasia. Several characters place *G. carolinii* within the Tetanurae³, and closer to Neotetanurae⁴ than to Torvosauroidae⁴. *G. carolinii* is the largest theropod ever recorded from the Southern Hemisphere, and is probably the world's biggest predatory dinosaur, having a body 12.5 metres long and an estimated weight of 6 to 8 tonnes.

Order Scurischia
Suborder Theropoda
Infraorder Tetanurae

Giganotosaurus carolinii gen. et. sp. nov.

Holotype. MUCPv-CH-1 (Museo de la Universidad Nacional del Comahue, El Chocón collection), a disarticulated skeleton represented by a partial skull, most of the vertebral column (most of the presacral column is currently in plaster), complete pectoral and pelvic girdles, both femora, left tibia and fibula.

Etymology. *Gigan*, giant (Latin); *notos*, austral (Greek); in reference to its enormous size and latitudinal provenance; *saurus*, reptile (Greek); *carolinii*, in honour of Rubén D. Carolini, who found the specimen.

Horizon and locality. Candeleros Member, Río Limay Formation (Albian-Cenomanian) Neuquén Group, 15 km south of Villa El Chocón, Neuquén Province, Argentina. The Río Limay Formation, primarily exposed in the eastern part of Neuquén Province, has yielded sauropods^{5,6}, theropods⁷ and dinosaur footprints⁸.

Diagnosis. *G. carolinii* has the following characters: dorsoventrally wide main body of the maxilla, with subparallel dorsal and ventral edges; eave-like supraorbital lacrimal-postorbital contact (preliminary considered convergent with *Abelisaurus*⁹); two pneumatic foramina in the internal side of the quadrate; symphyseal end of the dentary dorsoventrally expanded, bearing a ventral process; proximal end of the scapula forwardly pro-

jected above the coracoid; tubercle-like insertion for triceps in the ventral border of the scapula; lobule-shaped obturator process of the ischium; dorsally projected femoral head; and posterior intercondylar groove in the proximal end of the tibia. **Description.** The maxilla is long and high. It has a pronounced subnasal process and a small, elliptical maxillary fenestra as in *Allosaurus*¹⁰ and *Tyrannosaurus*¹ (Fig. 1a). The lacrimal has a rugose, posterodorsally oriented crest. The postorbital has an anteroventrally projected jugal process, broader anteroposteriorly than transversely. This process projects anteriorly into the orbit as in *Tyrannosaurus*¹, *Abelisaurus*⁹ and *Carnotaurus*¹¹. The quadrate is dorsoventrally long (Fig. 1b) and the dentary has a dorsoventrally expanded proximal end resembling that of *Piatnitzkysaurus*¹² (Fig. 1c). The neck was strong and powerful. The axis has a robust centrum and odontoid process. The posterior cervicals have short centra with pleurocoel subdivided by a lamina (as in *Tyrannosaurus*; P. Currie, personal communication). These centra are ventrally flat and have subhemispherical anterior articulations. The dorsals have high neural arches and deep pleurocoels. The caudals have posterodorsally elongate neural spines and robust centra, the transverse processes are anteroposteriorly long, laterodorsally and posteriorly oriented. Proximal chevrons are blade-like and distally expanded in lateral view. The pectoral girdle (Fig. 2) is proportionally smaller than in *Tyrannosaurus*¹ (scapula/femur length ratio less than 0.5). The scapular blade has parallel borders, with a strong tubercle for the triceps insertion, and a transversely thickened distal end. The coracoid is small and hook-shaped with an externally open coracoid foramen. The ilium (Fig. 2) has a convex dorsal border, low postacetabular blade and a narrow brevis-shelf as in *Allosaurus*¹⁰ and *Tyrannosaurus*¹. The pubis possesses an open obturator notch and a well-developed pubic foot, shorter anteriorly than posteriorly as in *Allosaurus*¹⁰. The ischium is straight, distally expanded, with a lobulate obturator process. The femur is sigmoidal in lateral view. It has an extremely robust head, with a deep posterior sulcus. The wing-like lesser trochanter is placed well below the greater trochanter, which is short anteroposteriorly. A weak trochanteric shelf is present. The fourth troch-

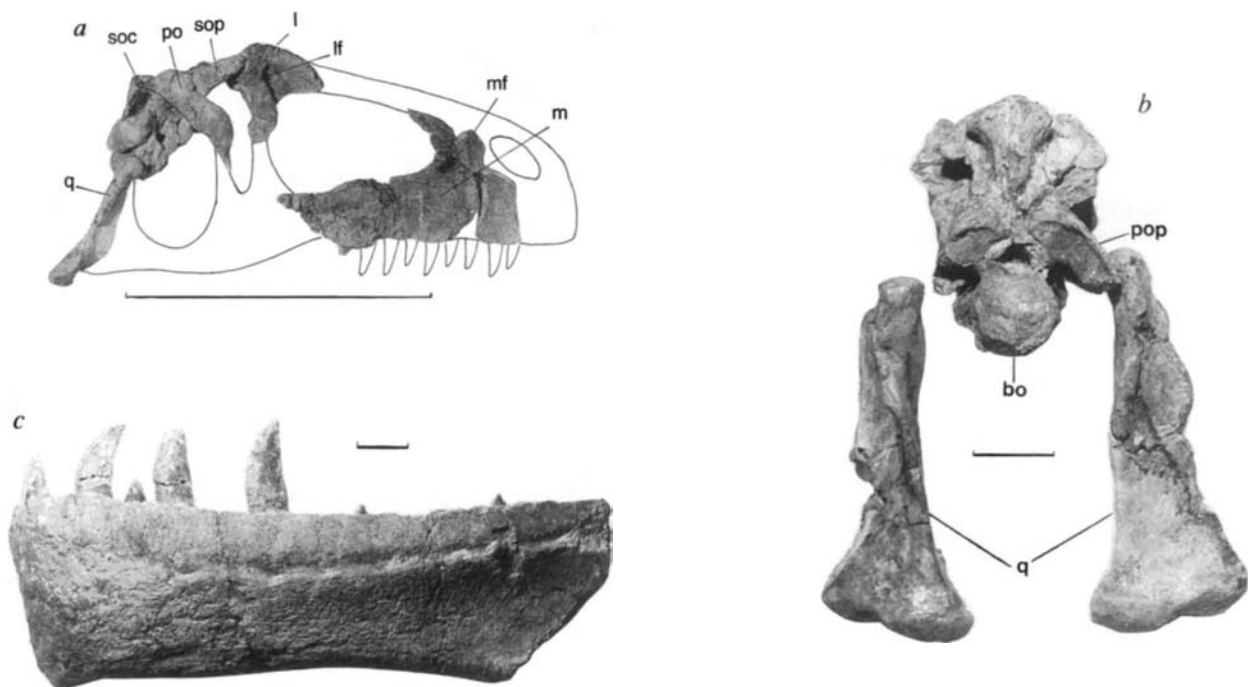


FIG. 1 a, Skull of *Giganotosaurus carolinii*. Abbreviations: l, lacrimal; lf, lacrimal foramina; m, maxilla; mf, maxillary fenestra; po, postorbital; q, quadrate; soc, supraoccipital; sop, supraorbital process. Scale bar, 1 m.

b, Braincase and quadrates in posterior view. Abbreviations: bo, basioccipital; pop, paraoccipital process; q, quadrates. Scale bar, 10 cm. c, Left dentary in lateral view. Scale bar, 10 cm.



FIG. 2 Skeletal restoration of *Giganotosaurus carolinii*, shown with man to indicate size.

anter is large, posteriorly projected and proximally placed. The tibia has an expanded proximal end with a wide articular surface. The shaft is compressed anteroposteriorly, and the distal edge is sigmoidal in anterior view. There is no fusion with the proximal tarsals. The facet for the ascending process of the astragalus shows the same grade of development as in *Allosaurus*¹⁰, *Piatnitzkysaurus*¹² and *Torvosaurus*¹³ (more than 20% tibial length).

Many unequivocal synapomorphies indicate that *Giganotosaurus* is closer to tetanurans than to ceratosaurs and more basal theropods (Fig. 3). *Giganotosaurus* shares with the Tetanurae³ (node 1) a tibia with fibular crest, an anterior tubercle in the fibula, and an ascending process of the astragalus more than 20% of the tibial length. Furthermore, *Giganotosaurus* and the Neotetanurae⁴ (node 2) share a strong and laterodorsally pointed lacrimal crest, a pubis with opened obturator foramen, a pronounced pubic foot, a femur with a medially oriented head, and an anterior intercondylar groove on the distal end of the femur. Nevertheless, retention in *Giganotosaurus* of an ischium longer than two-thirds the length of the pubis, a distally expanded ischium, a non-triangular obturator process of the ischium, and an ascending process of the astragalus of less than 25% of the tibial length, precludes its inclusion within the Coelurosauria¹⁴. The most impressive characteristic of *Giganotosaurus* is its enormous size. With a skull of about 1.53 m in length roughly twice the size of that of *Abelisaurus*⁹ (85 cm) and *Allosaurus*¹⁰ (75 cm), *Giganotosaurus* is one of the largest known theropods.

The only theropod that is similar in size to *Giganotosaurus* is *Tyrannosaurus rex*¹, the largest specimen of which is informally known as 'Sue' (ref. 15). The disarticulated cranial bones of *Giganotosaurus* (Fig. 2) do not allow accurate size comparisons with 'Sue', but the femur of *Giganotosaurus* (1.43 m in length) is about 5 cm longer than that of 'Sue'. Furthermore, the more robust bones of *Giganotosaurus* indicate that it was heavier than *Tyrannosaurus*.

Patagonian records of gigantic dinosaurs also include sauropods. The largest of these is *Argentinosaurus huinculensis*⁶, a colossal titanosaur with dorsal vertebrae 150 cm in height, which was found in higher sections (Huincul Member) of the Rio Limay formation.

Although large sauropods are not known from the slightly older Candeleros Member, sauropod remains are abundant in the floodplain deposits¹⁶ that entombed *Giganotosaurus*. These medium-size (13 m long) sauropods include the basal titanosaur *Andesaurus delgadoi*⁵ and an as yet unnamed diplodocid-like taxon¹⁷. The discovery of *Giganotosaurus* sheds light on the trophic relationships of the megavertebrate assemblage in the early Upper Cretaceous of Patagonia.

Giganotosaurus emphasizes the large diversity of South American theropods and, along with other findings (such as *Afrovenator*⁴), shows that basal tetanurans inhabited Gondwana during the Cretaceous. That the enormous size of *Tyrannosaurus* and *Giganotosaurus* evolved independently suggests that gigantism may be linked to common environmental conditions of their ecosystems. □

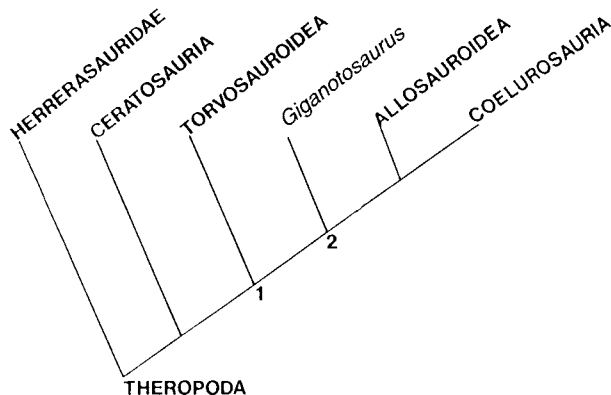


FIG. 3 Cladogram showing the phylogenetic position of *Giganotosaurus carolinii*.

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Polyphenol control of nitrogen release from pine litter

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THE importance of dissolved organic nitrogen in ecosystem nutrient fluxes and plant nutrition is only beginning to be appreciated^{1,2}. Here we report that the polyphenol concentration of decomposing *Pinus muricata* litter controls the proportion of nitrogen released in dissolved organic forms relative to mineral forms (NH_4^+ + NO_3^-). We have previously shown that concentrations of polyphenols in *P. muricata* foliage vary along an extreme soil acidity/fertility gradient³. Apparently this feedback to soil conditions controls the dominant form in which litter nitrogen is mobilized, facilitating nitrogen recovery through pine-mycorrhizal associations, minimizing nitrogen availability to competing organisms, and attenuating nitrogen losses from leaching and denitrification. Polyphenol control of nitrogen dynamics helps explain the convergent evolution of tannin-rich plant communities on highly leached soils.

Plant communities adapted to strongly acidic and infertile soils can sustain productivity despite low nitrogen availabilities and high potential for nitrogen loss from the ecosystem⁴. Mineralization (conversion of nitrogen from organic forms to NH_4^+ and NO_3^-) is generally considered to control the bioavailability and mobility of nitrogen released from decomposing litter⁵. There is debate as to whether measured rates of nitrogen mineralization in the litter layers of ecosystems on strongly acidic and infertile soils (such as coniferous forests or heathlands) are sufficient to account for vegetation uptake, particularly given the high rates of root turnover⁶⁻⁸. Whether the concentration of nitrogen, lignin, polyphenols or some combination of these is the litter quality factor that controls nitrogen release is also debated⁹⁻¹¹. Although nutrient cycling studies in forest ecosystems tend to focus only on mineral forms of available nitrogen, where efforts have been made to speciate forms of nitrogen released from litter, dissolved organic nitrogen (DON) is found to be the dominant vehicle of nitrogen transport^{1,12}. Fractionation reveals that most of this DON is not comprised of free amino acids or proteins but rather is associated with humic substances such as protein-tannin complexes¹. The distinction between litter nitrogen released as ammonium, nitrate or some form of DON is important because the form in which nitrogen is mobilized determines which organisms can utilize it and the potential for its loss owing to leaching or denitrification.

The Ecological Staircase, in northern California, is a series of coastal terraces that together make an extreme edaphic gradient. It includes pygmy forest communities that have been supported over geological time on intensely leached soils that are extremely acidic and nitrogen-limited, with negligible inputs or losses of nutrient elements^{13,14}. In these ecosystems, the small remaining pools of nutrients are contained entirely in the biomass and humus¹³. *Pinus muricata* occurs on the ancient and extremely infertile soil of the pygmy forest, on moderately acidic and infertile soils under tall pine forests, and also on slightly acidic and highly fertile soils at the margins of the coastal prairie. To understand the adaptations that sustain pine productivity, a study of nitrogen mobilization and litter quality was performed on litter of *P. muricata* from these three distinct edaphic conditions.

Pine-litter nitrogen mineralization rates during a three-week aerobic incubation vary more than tenfold among samples, but net release rates of dissolved organic nitrogen vary twofold in

the opposite direction (Fig. 1). Mineral nitrogen was released almost entirely as NH_4^+ rather than NO_3^- . Release rates of both mineral and organic forms of nitrogen are significantly correlated ($P < 0.001$) with litter polyphenol concentrations. There are highly significant correlations ($P < 0.001$) between the ratio of DON:mineral nitrogen and condensed tannin concentrations ($r^2 = 0.99$), total phenolic concentrations ($r^2 = 0.90$), and litter carbon/nitrogen ratio ($r^2 = 0.76$), but not with lignin concentration (Fig. 2).

The importance of DON for litter nitrogen mobilization is indicated by the ratios of DON:mineral nitrogen, which can exceed 10:1. Variable release of mineral nitrogen from litter within species on contrasting sites has previously been reported^{6,7,15-17}, but these studies did not measure DON release. In the pygmy forest, measurement of mineral nitrogen alone would give a very misleading estimate of litter nitrogen mobilization.

Fractionation of dissolved organic matter in the incubation extracts (adjusted to pH 2.0) shows that over 60% of the DON occurs in the fractions containing protein-tannin complexes or free proteins¹ (hydrophobic organic acids + hydrophilic bases) (Table 1). However, less than 5% of the DON occurs in the free protein fraction (hydrophilic bases) when extracts are fractionated at ambient soil solution pH (~4.5). This difference suggests that DON is contained in protein-tannin complexes that become partly dissociated in acidified samples. Analysis of incubation extracts shows appreciable concentrations of soluble polyphenols (25–35 mg l⁻¹ tannic acid equivalent). More than 80% of the total phenolic content and approximately 50% of the dissolved organic carbon in these extracts occur in the hydrophobic organic acid fraction (Table 1).

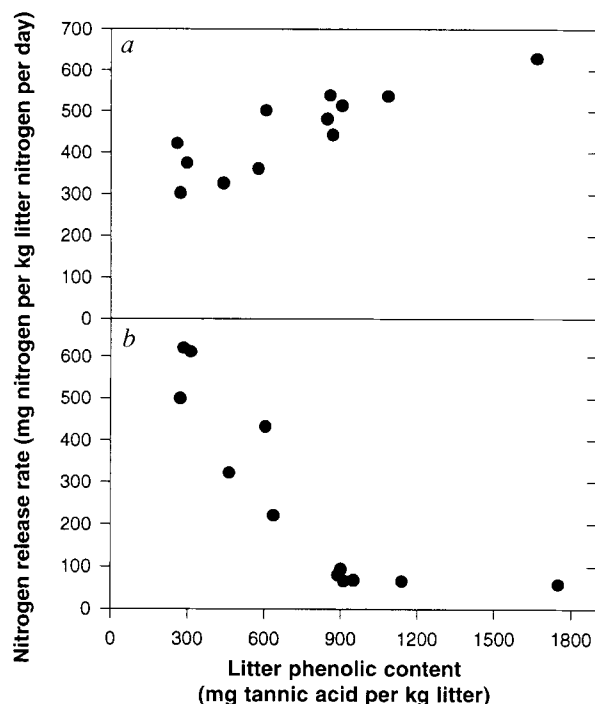


FIG. 1 *Pinus muricata* Oa (moderate to highly decomposed) litter nitrogen release versus concentration of total phenolics. a, DON; b, mineral nitrogen (NH_4^+ + NO_3^-). Litter under monospecies clusters of pine was sampled in three contrasting soil acidity/fertility conditions on the Ecological Staircase, near Mendocino, California. Values represent nitrogen release rates per g of litter nitrogen during a three-week aerobic incubation²⁶ under laboratory conditions at 25 °C. Mineral nitrogen (NH_4^+ + NO_3^-) in 1 M KCl extracts was measured conductimetrically²⁷, and DON was measured following persulphate oxidation²⁴. Total phenolic content (mg tannic acid equivalents per kg litter) was measured in 50% aqueous methanol extracts of Oa litter using the Prussian blue method²⁵.

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TABLE 1 Characterization of dissolved organic matter

Site	DON (hydrophobic) (%)	DON (hydrophilic bases) (%)	DON (hydrophilic acids) (%)	DOC (hydrophobic) (%)	Total phenols (hydrophobic) (%)
Coastal prairie margin	50	16	34	42	83
Tall pine forest	44	13	43	51	83
Pygmy forest	39	23	38	57	80

DON, DOC and total phenolic concentrations in litter incubation extracts were characterized by a resin-exchange fractionation procedure¹. The hydrophobic fraction (retained on XAD-8 resin) is dominated by high-molecular-mass phenolic acids including protein-tannin complexes; the hydrophilic bases fraction (retained on cation exchange resin, but not on XAD-8) is dominated by free proteins and amino acids; and the hydrophilic acid fraction (not retained on XAD-8 or cation exchange resin) is dominated by lower-molecular-mass 'fulvic' acids¹. Values represent the mean percentage ($n=3-6$) of the component contained in the designated fraction. DON concentrations were determined by persulphate oxidation followed by conductimetric assays²⁴; DOC concentrations by Dohrmann carbon analyser; and total phenol concentrations by the Prussian blue assay²⁵.

Comparing mean values between field sites, condensed tannin and total phenolic concentrations in current-year *P. muricata* foliage increase significantly ($P<0.05$) along the edaphic gradient (Fig. 3). Concentrations of mineral and organic forms of nitrogen released from *P. muricata* litter also vary appreciably (and inversely) between sites (Fig. 3). The observed variation in patterns of litter nitrogen release is interpreted as a feedback to phenotypic plasticity or genetic variation of foliar polyphenol concentrations.

Tannins form strong complexes with proteins that are sparingly soluble and recalcitrant to decomposition¹⁸. Protein-tannin complexes are very difficult to mineralize, but some ectomycorrhizal fungi associated with coniferous forests have been shown to utilize this nitrogen source¹⁹. Mycorrhizal *Pinus con-*

torta and the mycorrhizal fungi *Amanita muscaria* have been shown to utilize organic forms of nitrogen directly^{20,21}, and both species are common in the pygmy forest. Ericoid mycorrhizae have also been shown to produce extracellular enzymes that mineralize nitrogen from protein-tannin complexes²², and most of the plant species present in pygmy forest communities are ericaceous¹³. Direct utilization of organic nitrogen results in 'short-circuiting' of the nitrogen cycle where mineralization rates are not sufficient to supply plant uptake requirements²³.

Unlike nitrate, DON strongly adsorbs to soil surfaces, rarely leaches beyond the rooting zone⁷, and is not subject to gaseous loss by denitrification. Unlike ammonium, DON is not readily available to most soil organisms, and cannot easily be converted to a form that might be lost from the ecosystem. The impact of

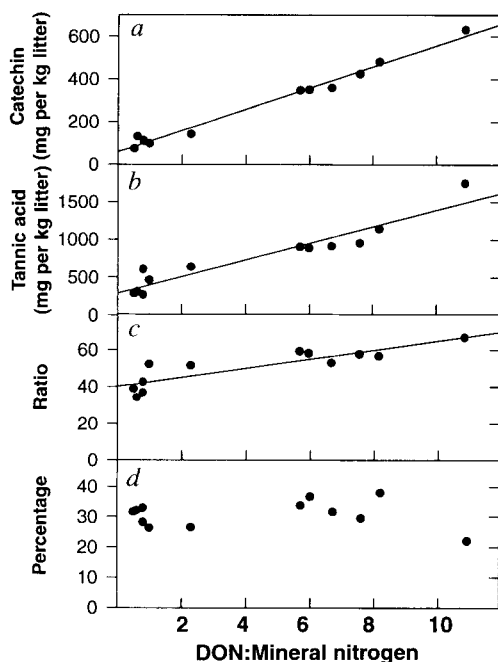


FIG. 2 *Pinus muricata* litter quality versus ratio of DON: mineral nitrogen released during a three-week aerobic incubation at 25 °C ($n=12$). a, Condensed tannins; b, total phenolics; c, C:N ratio; d, lignin. Condensed tannins were measured in aqueous methanol extracts as mg catechin equivalents per kg litter using the acidified vanillin assay²⁸; total phenolic content was measured as mg tannic acid equivalents per kg litter by the Prussian blue assay²⁵; C:N ratio was measured by a modified Kjeldahl digest²⁹ (N) and Leco furnace combustion (C); and lignin was measured as percent by the acid detergent method³⁰. Regressions of these parameters versus the ratio of DON: mineral nitrogen were as follows: condensed tannins ($r^2=0.99$, $P<0.001$), total phenolics ($r^2=0.90$, $P<0.001$), C:N ratio ($r^2=0.76$, $P<0.001$) and lignin (not significant).

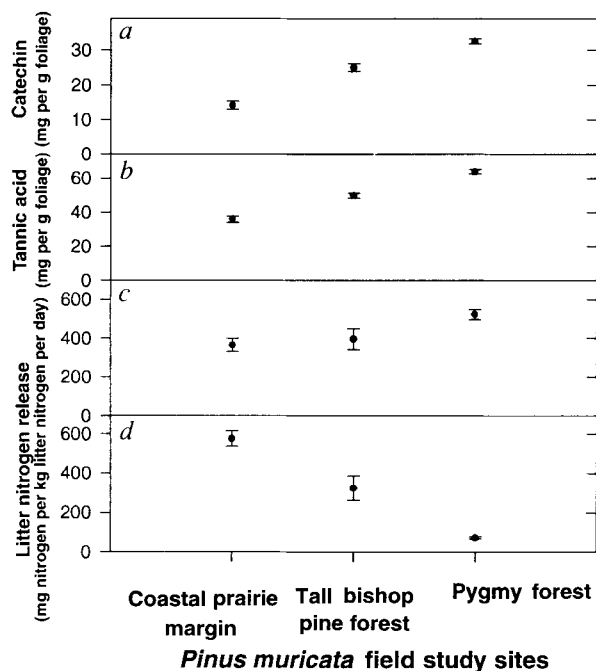


FIG. 3 *Pinus muricata* foliar phenolic concentration and litter nitrogen release in three contrasting soil environments of the Ecological Staircase. a, Foliar condensed tannins; b, foliar phenolic concentration; c, DON; d, mineral N ($\text{NH}_4^+ + \text{NO}_3^-$). Condensed tannins and total phenolics were measured in 50% aqueous methanol extracts of current-year *P. muricata* foliage collected from contrasting ecosystems of the Ecological Staircase soil chronosequence³ using the acidified vanillin²⁸ and Prussian blue²⁵ assays, respectively ($n>10$). Values are mg catechin equivalents per g foliage for condensed tannins, and mg tannic acid equivalents per g foliage for total phenolics. Mean rates of nitrogen release (g nitrogen released per kg litter nitrogen) during a three-week aerobic incubation at 25 °C of *P. muricata* Oa litter were measured conductimetrically²⁷ in raw samples (for $\text{NH}_4^+ + \text{NO}_3^-$) and following persulphate oxidation²⁴ for DON.

plant polyphenols on nitrogen cycling is interpreted as a plant adaptation to nitrogen limitation that effectively monopolizes the nitrogen contained in litter by immobilizing it into a form for which the plant's associated mycorrhizae have been shown to have a competitive acquisition advantage. As well as maximizing litter nitrogen recovery by the individual producer, this has consequences for sustaining long-term ecosystem productivity by minimizing nutrient losses. The convergent evolution of tannin-rich plant communities on strongly acidic and infertile soils throughout the world may be explained by this adaptation to nitrogen limitation. □

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Specification of anteroposterior cell fates in *Caenorhabditis elegans* by *Drosophila Hox* proteins

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ANTENNAPEdia class homeobox (*Hox*) genes specify cell fates in successive anteroposterior body domains in vertebrates, insects and nematodes^{1–3}. The DNA-binding homeodomain sequences are very similar between vertebrate and *Drosophila Hox* proteins, and this similarity allows vertebrate *Hox* proteins to function in *Drosophila*^{4–7}. In contrast, the *Caenorhabditis elegans* homeodomains are substantially divergent⁸. Further, *C. elegans* differs from both insects and vertebrates in having a non-segmented body as well as a distinctive mode of development that involves asymmetric early cleavages and invariant cell lineages. Here we report that, despite these differences, *Drosophila Hox* proteins expressed in *C. elegans* can substitute for *C. elegans Hox* proteins in the control of three different cell-fate decisions: the regulation of cell migration, the specification of serotonergic neurons, and the specification of a sensory structure. We also show that the specificity of one *C. elegans Hox* protein is partly determined by two amino acids that have been implicated in sequence-specific DNA binding. Together these findings suggest that factors important for target recognition by specific *Hox* proteins have been conserved throughout much of the animal kingdom.

The *C. elegans Hox* genes *lin-39* and *mab-5* are expressed and function in cells located in the mid and posterior body regions, respectively⁹. To determine whether their *Drosophila* homologues, *Sex combs reduced* (*Scr*) and *Antennapedia* (*Antp*) (see Fig. 1a), could substitute for the functions of *lin-39* and *mab-5*, we assayed three distinct cell fate choices: the positioning of migratory neurons along the anteroposterior body axis (Fig. 1b), the specification of serotonergic neurons (Fig. 1c), and the production of specialized sensilla called rays (Fig. 1d). The positioning of migratory neurons along the anteroposterior body axis is our most stringent test of *Hox* gene specificity because *lin-39* and *mab-5* have opposite effects on the same group of cells⁹. The left

TABLE 1 Rescue of *mab-5* and *lin-39* mutant phenotypes by expression of *C. elegans* and *Drosophila Hox* cDNAs

<i>Hox</i> protein	% of <i>mab-5</i> male sides with V rays* (n)	% of <i>lin-39</i> males with serotonin-immunoreactive CP neurons† (n)
None	0 (>100)	0 (>100)
<i>hs-mab-5</i>	44 (89)	0 (81)
<i>hs-Antp</i>	13 (77)	1.2 (83)
<i>hs-lin-39</i>	2.4 (85)	24 (72)
<i>hs-Scr</i>	0 (74)	4.3 (69)

* The average number of V rays per rescued side was 1.5 for *hs-mab-5*, 1.9 for *hs-Antp* and 1 for *hs-lin-39*. The left and right sides of the tail were scored independently.

† The average number of serotonergic CP neurons per rescued animal was 1.4 for *hs-lin-39* and 1 for *hs-Antp* and *hs-Scr*.

and right Q neuroblasts (QL and QR) are born in equivalent anteroposterior positions, but they and their descendants migrate in different directions to either posterior (QL) or anterior (QR) body regions¹⁰ (Fig. 1b). The anterior migration of the QR descendants is dependent on *lin-39* (refs 11, 12), and the posterior migration of the QL descendants is dependent on *mab-5* (ref. 13). Expressing an *hsp-16 (hs)-mab-5* complementary DNA gene fusion in a *mab-5* mutant can cause both the QL and the QR descendants to behave like wild-type QL descendants¹⁴: two cells, Q.paa and Q.pap, stop their anterior migration (Fig. 2), and Q.ap migrates posteriorly. Similarly, we find that expressing *hs-lin-39* in *lin-39* mutants can rescue the anterior migration of the QR descendants (Fig. 2) and cause an anterior shift in the final positions of the QL descendants (data not shown). Thus *hs-lin-39* and *hs-mab-5* have opposite and specific effects on the direction of migration of the Q-cell descendants.

To determine whether the *Drosophila Hox* proteins could substitute for their *C. elegans* homologues in directing these migrations, we expressed *hs-Scr* and *hs-Antp* cDNA gene fusions in *lin-39* and *mab-5* mutants and determined the final positions of the Q.paa and Q.pap cells. We found that, like *hs-lin-39* expression, *hs-Scr* expression in *lin-39* mutants could promote the anterior migration of the QR and QL descendants (Fig. 2 and data not shown). Thus the specificity of *hs-Scr* function in the Q-cell descendants is the same as that of *hs-lin-39*. Similarly, *hs-Antp* expression, like *hs-mab-5* expression, inhibited the anterior migration of both the QL and QR descendants in *mab-5* mutants (Fig. 2), and could also cause Q.ap to migrate posteriorly (not

shown). Thus the specificity of function of the *Drosophila* and *C. elegans* *Hox* proteins is conserved in this sensitive and specific assay, the control of anterior versus posterior migration of the Q-cell descendants.

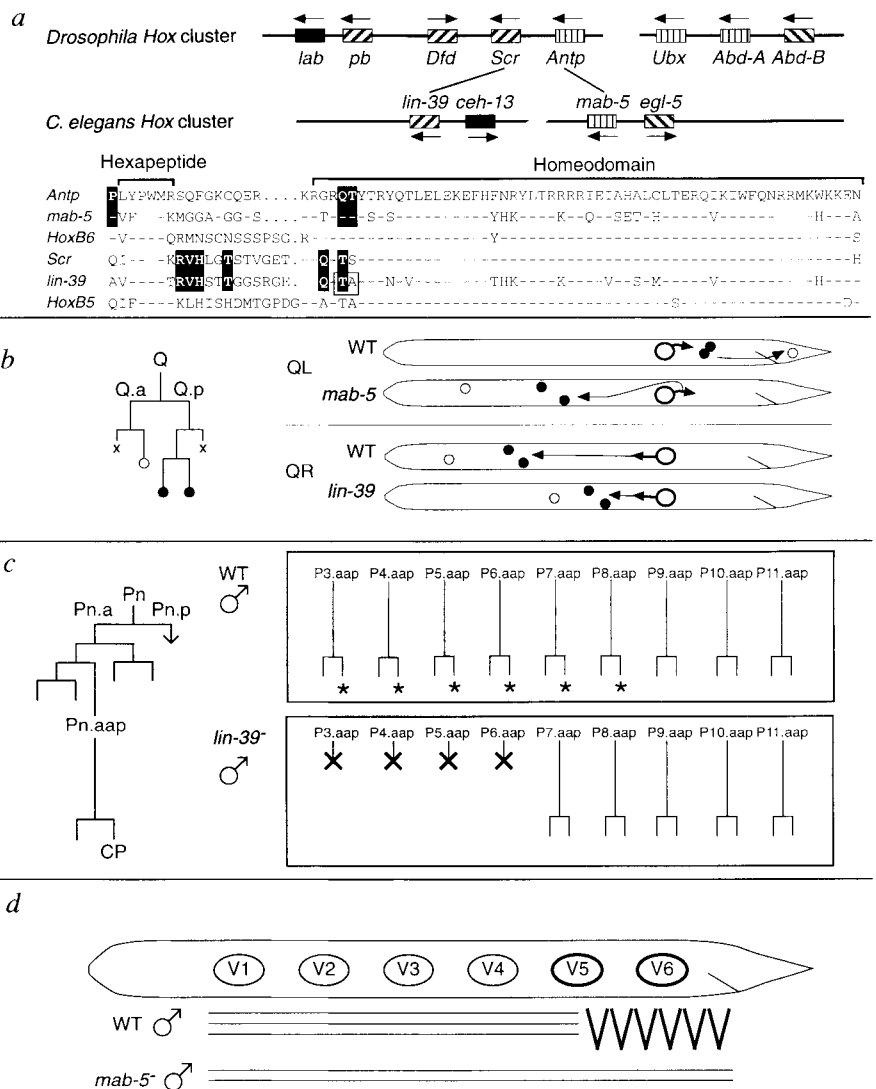
To determine whether the conserved functional specificity of these proteins extends to other cell-fate decisions, we also examined the production of serotonergic neurons in the mid-body region which are specified by *lin-39* (Fig. 1c) and the production of rays in the posterior-body region which are specified by *mab-5* (Fig. 1d). We found that *hs-lin-39*, *hs-Scr* and, in one animal, *hs-Antp* expression could restore serotonin immunoreactivity to *lin-39*(-) ventral-cord neurons (Fig. 3a, b; Table 1). Thus, in this cell type, function has been conserved but specificity has not. We next examined the *mab-5*-dependent production of rays in the posterior-body region (Fig. 3c, d). We found that both *hs-mab-5* and *hs-Antp* expression could restore ray development in *mab-5*(-) males but that *hs-Scr* expression could not (Fig. 3e, f; Table 1).

How is the specificity of these proteins achieved? The sequence similarity of these functionally conserved homologues is limited

to the homeodomain and a short sequence just amino-terminal to the homeodomain (highlighted in Fig. 1a). To determine whether these residues are important for the functional specificity of the *C. elegans* *Hox* proteins, we changed two homeodomain amino acids in *lin-39* (boxed in Fig. 1a) to those present in both *mab-5* and *Antp*, and investigated whether the modified *lin-39* protein (*hs-lin-39QT*) now had *mab-5*-like activity. We found that *hs-lin-39QT* retained some *lin-39* function and could cause an anterior shift in the final positions of the QR descendants in *lin-39* mutants (Fig. 2). However, *hs-lin-39QT*, like *hs-mab-5*, could also cause a posterior shift in the final positions of the QL descendants in *mab-5* mutants (Fig. 2). Therefore, these two amino acids do have a role in determining the functional specificity of the *C. elegans* *Hox* proteins. Studies in *Drosophila* show that N-terminal homeodomain amino acids are also critical to *Drosophila* *Hox* protein specificity^{15,16}, and that the identity of these amino acids can influence the *in vitro* DNA binding specificity of *Drosophila* homeodomains¹⁷. Thus specific enhancer sequences may have been conserved between flies and worms.

FIG. 1 *Hox* gene homologies and selected *C. elegans* *Hox* mutant phenotypes. a, The *Drosophila* and *C. elegans* *Hox* clusters and alignment of fly, nematode and mouse homeodomains and hexapeptide amino acids⁸. The *lin-39* homeodomain is most similar to *Pb* (43 of 60 identical homeodomain amino acids), *Dfd* (46 of 60) and *Scr* (46 of 60); *mab-5* is most similar to *Antp* (44 of 60), *Ubx* (43 of 60) and *Abd-A* (43 of 60); and *egl-5* and *ceh-13* are homologous to *Abd-B* (32 of 60) and *lab* (41 of 60), respectively. Based on amino acid identities in and near the homeodomain¹¹, we have chosen *Scr* and *Antp* as the closest *lin-39* and *mab-5* homologues, respectively. The complete *Antp* amino acid sequence for the conserved regions is shown; identical aligned amino acids are represented by dashes, and gaps by dots. Amino acid identities among the functional pairs are highlighted.

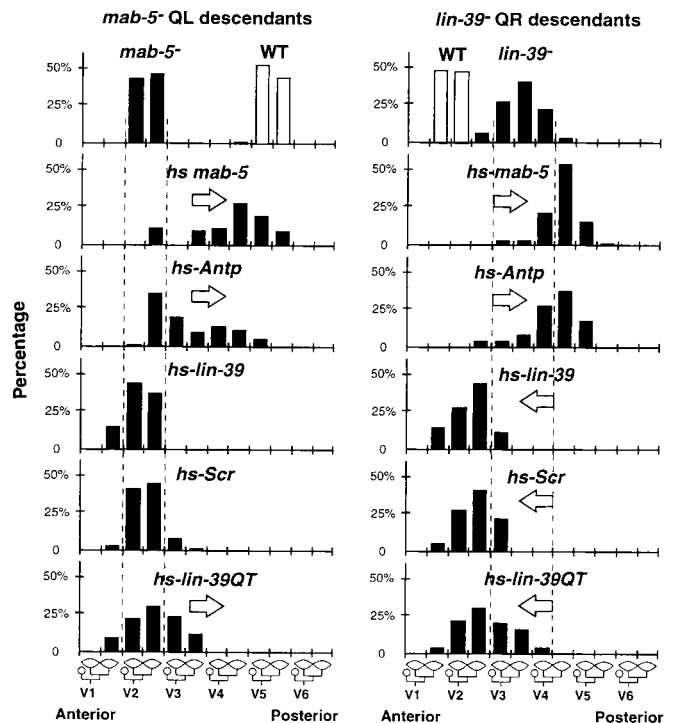
b, The cell lineage and migration of the Q neuroblasts and their descendants in wild-type, *mab-5*, and *lin-39* mutants¹⁰⁻¹³. The left and right Q cells (QL and QR) follow identical cell lineages, producing three neurons (open and filled circles) and two cell deaths (crosses). At hatching the Q cells (large open circles) are each in approximately the same anteroposterior position. A few hours after hatching, the QL cell migrates posteriorly a short distance and divides. One descendant, QL.ap, migrates into the tail (open circle) but the two surviving descendants of the posterior cell (filled circles) do not migrate substantially. Similarly, soon after hatching, the QR cell migrates anteriorly a short distance and divides. All its descendants continue to migrate towards its positions. In *mab-5* mutants, the QL cell still migrates posteriorly, but its descendants migrate anteriorly like the wild-type QR-cell descendants. In *lin-39* mutants, the QR descendants stop migrating prematurely. The QL-cell descendants are not affected. c, *lin-39*-dependent development of ventral P cells. Each P cell (Pn) divides several times during the first larval stage, producing multiple neuronal and epidermal cells; these are named according to position in the lineage ('a' for anterior, 'p' for posterior)¹⁰. In males, the P3.aap to P11.aap cells divide during the third larval stage. The posterior daughter cells, Pn.aap, are the CP neurons, CP1 to CP9. In wild-type males, CP1-CP6 stain with anti-serotonin antibodies¹⁸ (asterisks). In *lin-39* mutant males, the P3.aap to P6.aap cells undergo programmed cell death (cross) during the first larval stage, and none of the remaining five CP neurons stain with anti-serotonin antibodies^{12,19}. d, *mab-5*-dependent ray development. In males, the



anterior V cells produce adult epidermal structures called alae (ridges in the cuticle symbolized by parallel lines) and the posterior V cells, V5 and V6, produce sensory organs called rays^{10,20} (symbolized by spikes). The production of rays by the V5 and V6 cells is dependent on *mab-5* function; in *mab-5* mutants the V5 and V6 cells make alae, as do the anterior V cells¹³.

FIG. 2 Positioning of migratory QL and QR descendants. The position of the Q.paa and Q.pap cells (filled circles in Fig. 1b) relative to the positions of the V-cell descendants Vn.pa and Vn.pp after about 22 h development is shown. The Q.ap cells (open circles in Fig. 1b) were not assayed, because their final positions following expression of the *Hox* proteins are quite variable and they often lie in neuron-rich regions, making them difficult to identify. The migrations are normally complete by 10 h¹⁰. The positions of these QL and QR descendants in the wild type (WT) are shown in the white bars, which represent the percentage of total Q-cell descendants scored. The positions of these QL and QR descendants in *mab-5* and *lin-39* mutants or in *Hox* mutants following induced *Hox* gene expression are shown in black. The broken lines indicate the positions of the Q-cell descendants without heat shock. Anterior and posterior shifts of position are indicated by arrows. The final positions of the Q-cell descendants are not affected in either non-heat-shocked animals containing the constructs or in heat-shocked animals not containing the constructs. A minimum of 50 cells (two per side) were scored in all cases.

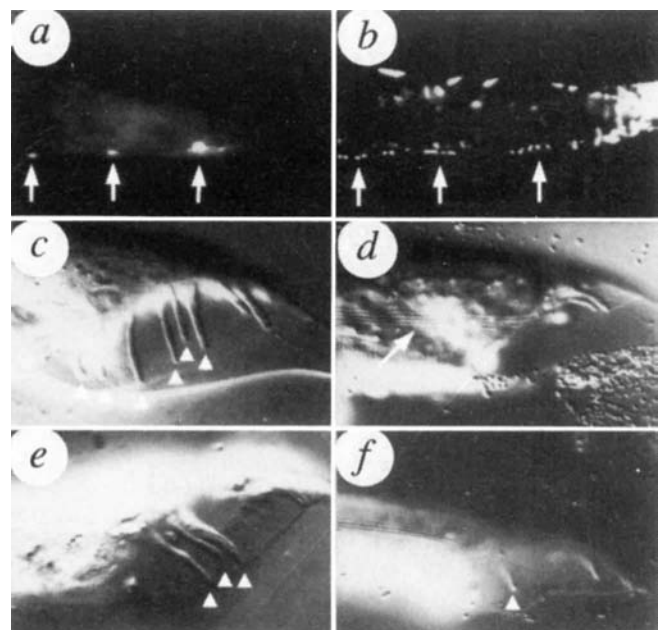
METHODS. The *hs-mab-5* construct has been described previously¹⁴. To construct *hs-lin-39*, *hs-Scr* and *hs-Antp*, primers homologous to the start and stop codons of each gene were used to amplify the coding sequence from cDNA clones and the sequenced amplification products were ligated into the *Hpa*I site of the *hsp16.1* gene²¹. Each construct (20 mg ml⁻¹) was co-injected with the *dpy-20(+)* plasmid pMH86 (80 mg ml⁻¹) into *dpy-20(e1282)* hermaphrodites²². Integration of the *dpy-20(+)* *hs-Hox* arrays was induced by γ -ray mutagenesis (C. Kari, A. Fire and R. K. Herman, personal communication) and independent lines containing integrated arrays were isolated (*mulS20*=*hs-mab-5*; *mulS21*, *mulS22*=*hs-Antp*; *mulS23* (spontaneous integration), *mulS24*=*hs-lin-39*; *mulS25*=*hs-Scr*). These integrated arrays were crossed into *mab-5(e1239)* and *lin-39(mu26)* mutant strains. The *hs-lin-39QT* construct was made by digesting the *hs-lin-39* construct with *Bst* U1, which cuts the DNA just 5' of the homeodomain, and then amplifying the gene with the same start and stop codon primers along with an oligonucleotide that hybridizes to sequences on both sides of the *Bst* U1 site and introduces the mis-sense base changes. Sequenced amplification products were ligated into *hsp16.1*; *hs-lin-39QT* was co-injected with pMH86 (*dpy-20(+)*) into *dpy-20(e1282)*. Lines segregating the array were crossed into *mab-5(e1239)* and *lin-39(mu26)* mutant strains; only non-Dpy animals were scored. To induce heat-shock expression, animals on Petri plates were placed at 31 °C for 30 min and then returned to 20 °C. Heat-shock-induced expression of these



constructs at the developmental stages that effect the positioning of the Q cells, the production of rays, or the induction of serotonin synthesis in the CP neurons, does not cause other obvious transformations. To influence positioning of the Q neuroblast descendants, larvae 3–5 h old (after hatching) were heat-shocked. At 20–24 h after hatching, the positions of the Q descendants, Q.paa and Q.pap, were recorded in reference to the V1–V6 seam cell progeny Vn.pp and Vn.pa. The Q.ap cell, which is normally in the head or tail, was often found in the body region (data not shown).

FIG. 3 Rescue of *lin-39* and *mab-5* phenotypes by *hs-Hox* gene expression. Anti-serotonin (a) and DAPI staining (b) in *lin-39* mutant males following expression of *hs-lin-39*. Serotonin is not expressed in any ventral cord neurons in *lin-39* mutant males^{12,19}. Expression of *hs-lin-39*, *hs-Scr* and *hs-Antp* resulted in one or more serotonin-immunoreactive neurons (arrows) with posteriorly directed processes in the ventral cord (Table 1). The cell bodies of these serotonin-immunoreactive neurons were on the left side of the ventral cord, consistent with these cells being the surviving CP neurons. The CP neurons derived from P7 and P8 expressed serotonin most frequently. c, Wild-type male tail showing six V rays (arrowheads) and three T rays. d, *mab-5* mutant male tail; the posterior V cells produce anterior structures called alae (arrow) instead of rays. The T rays are not affected by *mab-5* mutations. Ray production is rescued in *mab-5(-)* mutants by *hs-mab-5* (e) and *hs-Antp* (f) expression. The *hs-mab-5* and *hs-Antp* rescued animals produce similar numbers of rays (Table 1); *hs-lin-39* expression could also restore ray development, but to a much lesser extent (Table 1). This is consistent with the observation that *lin-39* function promotes the production of rays in some mutant backgrounds (L. Wrischnik and C.K., in preparation).

METHODS. To induce serotonin synthesis in CP neurons in *lin-39* mutants, 30–35 h larvae (20 °C) were heat-shocked as described in Fig. 2 and returned to 20 °C until many of the hermaphrodites were producing eggs. The adults were then collected, fixed and stained for serotonin immunoreactivity¹⁸. Only animals with strong immunostaining of the NSM and other head and posterior ganglia neurons were scored for immunoreactive CP neurons. Heat-shock treatment alone did not affect CP cell development. The data for serotonin-immunoreactive CP neurons in Table 1 was generated both from the strains described in Fig. 2 and from similar strains carrying non-integrated arrays of the *hs* constructs and the dominant co-transformation marker *rol-6(su1006)*²³. Only *Roller* males were scored in these non-integrated lines. To induce ray production in *mab-5* mutant males, larvae 10–15 h old (20 °C) were



heat-shocked and returned to 20 °C. Late L4 to early-adult males were scored by Nomarski differential interference contrast microscopy for the production of V-cell-derived rays. Only animals with morphologically distinct rays were scored as positive. Each side was scored independently. Heat-shock treatment alone did not result in ray production.

In summary, we have found that the *Drosophila* *Hox* proteins *Scr* and *Antp* can substitute for the normal function of their *C. elegans* homologues, *lin-39* and *mab-5*, in three different cell-fate decisions. Furthermore, the specificity of their function has been remarkably well conserved, especially in directing the migration of the Q-cell descendants. These findings, coupled with previous findings that vertebrate *Hox* proteins can function in *Drosophila*⁴⁻⁷ suggests that factors important for target recognition by specific *Hox* proteins have been broadly conserved within the animal kingdom. □

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Failure of postsynaptic specialization to develop at neuromuscular junctions of rapsyn-deficient mice

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OF numerous synaptic components that have been identified, perhaps the best-studied are the nicotinic acetylcholine receptors (AChRs) of the vertebrate neuromuscular junction¹. AChRs are diffusely distributed on embryonic myotubes, but become highly concentrated (~10,000 μm^{-2}) in the postsynaptic membrane as development proceeds. At least two distinct processes contribute to this accumulation. One is local synthesis: subsynaptic muscle nuclei transcribe AChR subunit genes at higher rates than extrasynaptic nuclei, so AChR messenger RNA is concentrated near synaptic sites^{2,3}. Second, once AChRs have been inserted in the membrane, they form high-density clusters by tethering to a subsynaptic cytoskeletal complex. A key component of this complex is rapsyn, a peripheral membrane protein of relative molecular

mass 43K (refs 4, 5), which is precisely colocalized with AChRs at synaptic sites from the earliest stages of neuromuscular synaptogenesis⁶. In heterologous systems, expression of recombinant rapsyn leads to clustering of diffusely distributed AChRs, suggesting that rapsyn may control formation of clusters^{7,8}. To assess the role of rapsyn *in vivo*, we generated and characterized mutant mice with a targeted disruption of the *Rapsn* gene. We report that rapsyn is essential for the formation of AChR clusters, but that synapse-specific transcription of AChR subunit genes can proceed in its absence.

We replaced roughly 1 kilobase (kb) of the *Rapsn* gene⁹ with a neomycin-resistance gene by homologous recombination in embryonic stem ES cells (Fig. 1a). Two independent mutant ES-cell lines were injected into mouse blastocysts to generate germline chimaeras. Heterozygotes, which displayed no behavioural or histological abnormalities, were bred to obtain homozygotes. Homozygous mutants were born in expected numbers and were similar in appearance to normal littermates, but they died within hours after birth. Southern analysis confirmed that the gene had been disrupted (Fig. 1b), and immunoblotting confirmed the absence of rapsyn protein from mutant muscle (Fig. 1c). Dissection and conventional histological analysis revealed no detectable changes in internal organs, including in muscle, where rapsyn is most abundant, or heart and kidney, which express lower amounts of rapsyn¹⁰. Mutant mice breathed weakly, and were unable to support themselves on all fours or to lift their heads, suggesting that neuromuscular function was severely affected. Accordingly, we proceeded to characterize the neuromuscular junctions of rapsyn-deficient mice.

To assess the distribution of AChRs, we stained diaphragm muscles with a rhodaminated derivative of the selective, high-affinity ligand, α -bungarotoxin (BTX). In normal muscle, each muscle fibre is innervated at a single site near its midpoint, and bears a single synapse-associated cluster of AChRs (ref. 2 and Fig. 1f). Most of the synapses, and therefore most of the clusters, are located in an 'end-plate band' near the nerve trunk in the centre of the muscle (Fig. 1d). In mutant muscle, however, there were no detectable clusters of AChRs anywhere along the length of the fibres (Fig. 1e). Similarly, no AChR clusters were seen in mutants at embryonic day (E) 14, but were detected in littermate controls⁶. Thus, rapsyn is essential for AChR clustering.

Although we favoured the idea that rapsyn promoted AChR clustering directly, two indirect explanations were also possible. First, rapsyn deficiency could decrease AChR levels. To test this possibility, we used ¹²⁵I-labelled BTX to determine the number of AChRs in mutant muscle¹¹. Compared to controls, there was a ~60% increase in the amount of AChRs in mutant muscle by this measure, perhaps reflecting decreased fetal movement; activity normally suppresses AChR synthesis¹. Second, rapsyn could be required for the transmission of retrograde signals to the nerve, which in turn compromised their ability to induce AChR clustering. To test this possibility, we cultured myoblasts from mutants and wild-type littermates, allowing them to form myotubes, then stained them with rhodamine-BTX. Wild-type myotubes formed small numbers of AChR-rich 'hot spots', and this number was increased 20-fold following a 16-hour treatment with a recombinant fragment¹² of the nerve-derived clustering factor, agrin (Fig. 1h). Myotubes from rapsyn-deficient mutants were indistinguishable in shape and size from controls, but generated no 'hot spots', either spontaneously or in response to agrin (Fig. 1i). Together these results demonstrate that rapsyn induces AChR clustering by a cell-autonomous mechanism that does not involve AChR synthesis.

Even though AChRs did not form clusters in mutant muscles, they were concentrated in the central end-plate band (Fig. 1e). Analysis of single fibres showed that this non-uniformity reflected a gradient of AChR density in the membrane (Fig. 1g). This pattern suggested that AChR genes might still be transcribed preferentially by synaptic nuclei in mutant muscle,

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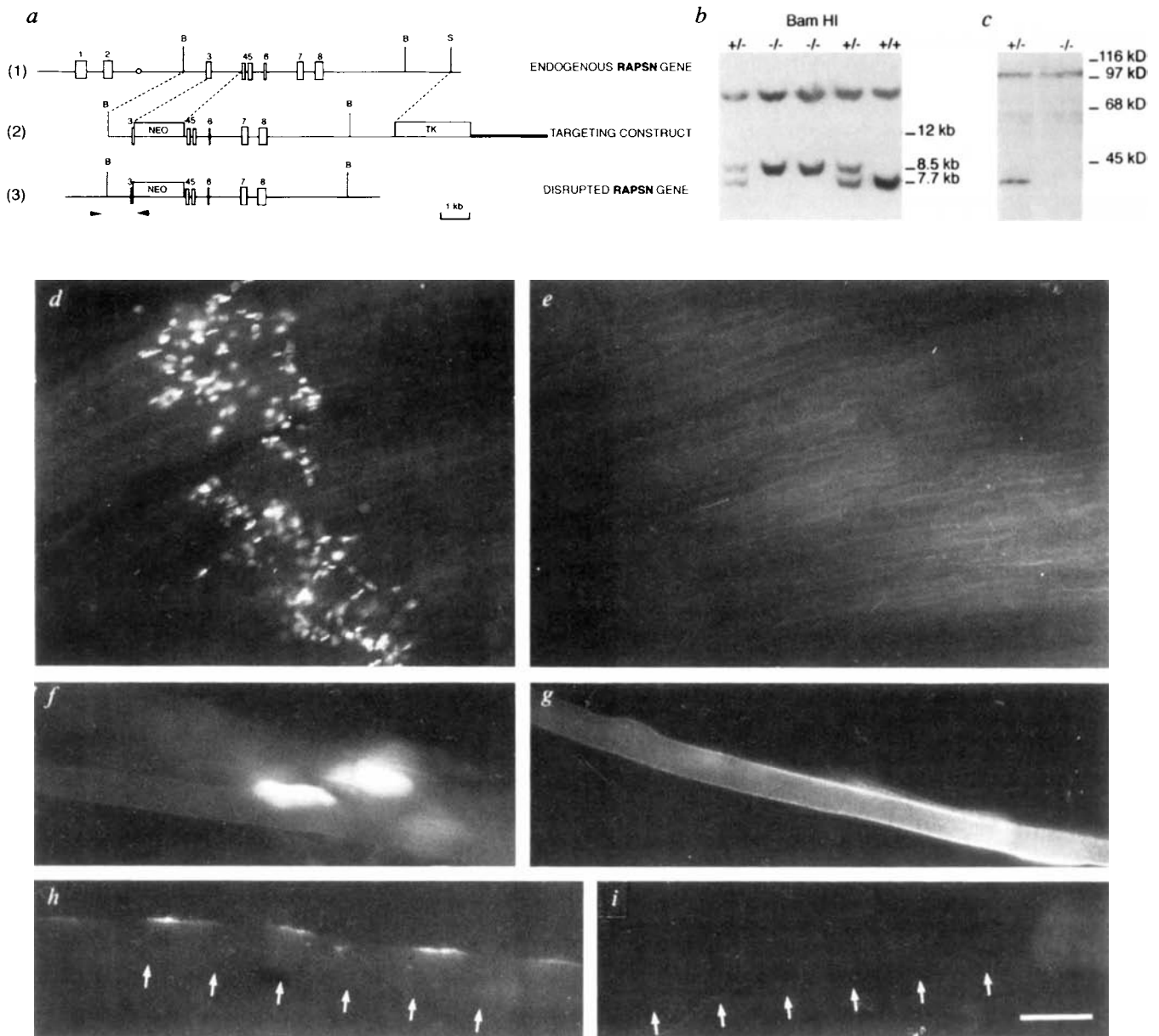


FIG. 1 Distribution of acetylcholine receptors in muscle fibres and cultured myotubes from rapsyn-deficient mice and controls (wild type and heterozygotes). **a**, Structure of the endogenous *Rapsn* gene (1), the targeting vector (2) and the disrupted gene (3). The targeting construct contained a deletion of ~1 kb of the gene including 119 bp of exon 3. **b**, Southern blot analysis of genomic DNA from mutant homozygotes and littermates. The 7.7-kb *Bam*HI fragment present in wild-type mice was altered to 8.5 kb in the mutants. **c**, Immunoblot of muscle protein extracts from mutant and control littermates. Antibodies to a C-terminal fragment of rapsyn recognized a 43K band in control but not in mutant extracts. **d-g**, PO diaphragms stained with rhodamine-BTX. Receptor clusters were distributed in the central region of normal diaphragms (**d**). Individual fibres teased from the muscle bore a single plaque-shaped cluster (**f**). Although mutant diaphragms lacked clusters, AChRs were concentrated in the central region of fibres (**e**, **g**). **h** and **i**, BTX-stained primary muscle cultures induced with recombinant agrin to form clusters of AChRs. Arrows mark myotube surface. Control myotubes formed AChR clusters (**h**), but myotubes cultured from rapsyn mutants failed to form any hotspots (**i**). Scale bar is 50 µm for **d** and **e**, and 10 µm for **f-i**.

METHODS. **a**, The targeting construct was generated by inserting *Rapsn* genomic fragments⁹ into cloning sites flanking the 'PGKNEO' cassette in the vector pPNT²⁵. Arrowheads indicate the primers used for PCR screening. The targeting construct was transferred to R1 ES cells by electroporation, and ~1% of (G418+FIAU)-resistant clones were homologous recombinants. **b**, Genomic DNA was digested with the restriction enzyme *Bam*HI and probed with a full-length rapsyn cDNA fragment⁵. **c**, Muscle protein extracts were fractionated by SDS-PAGE, and immunoblots were probed with 5943p, a C-terminal-specific rapsyn antibody⁸. **d-g**, Diaphragms were fixed for 1 h in 2% paraformaldehyde in phosphate-buffered saline (PBS), then incubated overnight with rhodamine-BTX in PBS. **h** and **i**, Primary muscle cultures were prepared on coverslips as described²⁶. Myotubes were treated for 16 h with medium from a cell line (gift from R. Scheller) that produces and secretes recombinant agrin of the x=12, y=4, z=8 form¹². Myotube cultures were then fixed and stained with rhodamine-BTX as described⁸. Mutants derived from two independent homologous recombinant ES cell lines were analysed and gave indistinguishable results throughout.

even though the AChRs subsequently failed to aggregate. The selective transcriptional potential of synaptic nuclei, first inferred from the concentration of AChR mRNA near synaptic sites², has been demonstrated directly by synaptic accumulation of reporter proteins in transgenic mice bearing promoter fragments of AChR subunit genes fused to a reporter gene³. We previously characterized mice in which sequences from the AChR 'adult' ϵ -subunit gene were linked to a nuclear-targeted β -galactosidase (*nlacZ*) gene, but these expressed *nlacZ* only postnatally¹³, whereas rapsyn mutants die on the day of birth (P0). To determine whether synapse-specific AChR gene transcription occurs in the absence of AChR clustering, we generated new transgenic mice in which *nlacZ* was controlled by a promoter fragment from the AChR 'fetal' γ -subunit gene. When transgenic mice with a stable insertion of this construct were stained for LacZ, we observed a zone of blue nuclei in the synaptic region of muscle fibres in neonates (Fig. 2a, c), a pattern similar to that previously described at later ages in AChR ϵ -*nlacZ* mice¹³. Identical patterns of staining were observed in homozygous rapsyn mutants that contained the γ -*nlacZ* transgene (Fig. 2b, d). Likewise, endogenous AChR α -subunit mRNA was concentrated at synaptic sites in both mutants and controls, as assessed by *in situ* hybridization (Fig. 2e–g), demonstrating that synapse-specific gene expression in the mutant was not unique to a single subunit or to a transgene. Thus selective transcription of AChR

subunit genes in synaptic nuclei occurs by a mechanism distinct from rapsyn-mediated AChR cluster formation. Moreover, this result suggests that the increased synaptic AChR density generated by this mechanism is sufficient to support neuromuscular transmission (neonatal movement) to a limited degree.

Although previous studies have implicated rapsyn only in AChR clustering, it seemed likely that the absence of rapsyn might perturb other synaptic components, either directly or indirectly. We used a battery of antibodies to synaptic components to compare the postsynaptic cytoskeleton, synaptic cleft and nerve terminals of mutants and littermate controls.

Both *in vivo* and in cultured myotubes, the AChR-associated cytoskeleton includes not only rapsyn but also several components originally identified as homologues and ligands of the spectrin-like protein, dystrophin¹⁴. These include the 400K dystrophin homologue, utrophin¹⁵; the syntrophins, a family of 58–59K proteins that associate with dystrophin and utrophin¹⁶, and dystroglycan¹⁹, a dimer of α - and β -subunits that binds to components of the basal lamina extracellularly and to utrophin intracellularly. *In vitro*, rapsyn links AChRs and dystroglycan²⁰. *In vivo*, rapsyn might attach AChRs to a preformed utrophin/syntrophin/dystroglycan complex or mediate assembly of the entire complex. To distinguish between these possibilities, we stained sections of wild-type and mutant muscles with antibodies to utrophin, α -dystroglycan, β -dystroglycan and α -syntrophin.

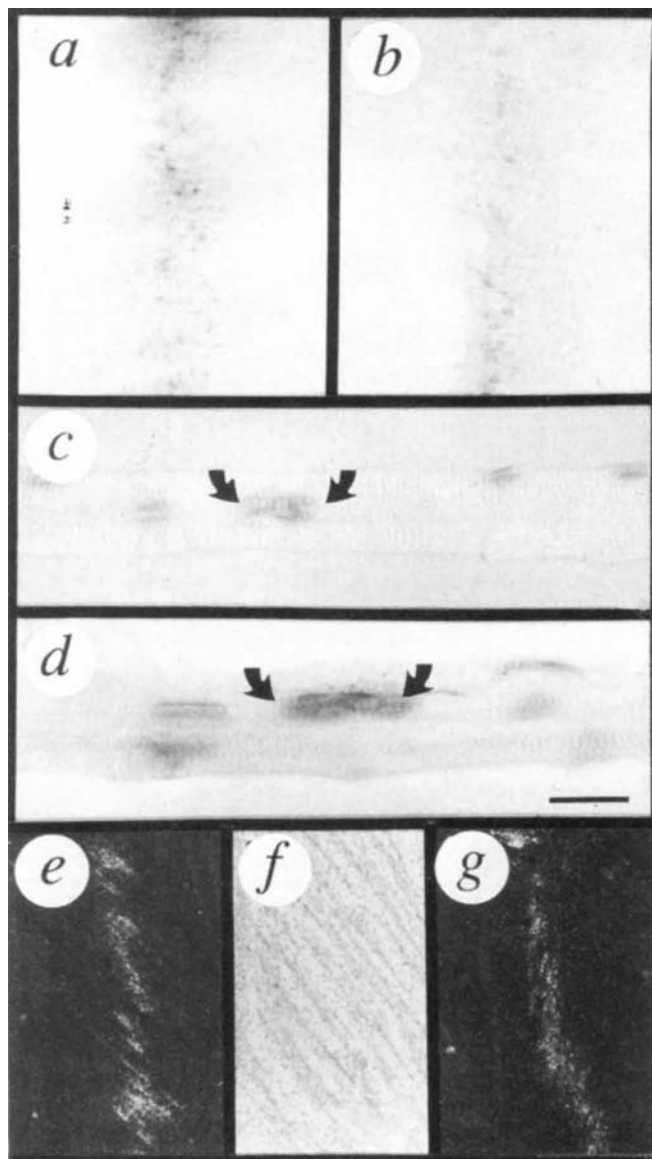


FIG. 2 AChR genes are selectively expressed by synaptic nuclei in the absence of AChR clusters. a–d, Rapsyn mutants with the γ -*nlacZ* transgene were generated by crossing the γ -*nlacZ* transgenic into a rapsyn-deficient line. Diaphragms of controls (a, c) and mutants (b, d) were stained for LacZ. In both, LacZ activity was confirmed to a zone in the central region of the muscle. In bundles of fibres teased from whole, fixed muscles, staining was strongest in the clustered synaptic nuclei (arrows in c and d). e–g, Intercostal muscles were processed for *in situ* hybridization, using a probe specific for the AChR α -subunit mRNA. In both controls (e) and mutants (g), AChR α -mRNA was concentrated in the central end-plate band. Positive and negative control probes showed uniform and undetectable labelling, respectively (not shown here but in ref. 27); e and g are dark-field micrographs; f is a bright-field micrograph of the same area shown in e. Scale bar is 150 μ m for a and b, 10 μ m for c and d, and 250 μ m for e and f.

METHODS. a–d, A promoter fragment from the AChR γ -subunit gene²⁸, extending from the *Ban*I site (+14 in relation to the transcription start site), to the *Bam*HI site 10 kb upstream, was used to direct the expression of a β -galactosidase gene with a nuclear localization signal (γ -*nlacZ*). Methods for generation and characterization of the transgenic mice are described in ref. 13. Mice containing a γ -*nlacZ* transgene were crossed to rapsyn heterozygotes, and the offspring were inbred to generate γ -*nlacZ*/rapsyn (+/–) and γ -*nlacZ*/rapsyn (–/–) mice, which were identified by PCR. Mice were fixed in paraformaldehyde, and stained for β -galactosidase activity as described in ref. 13. e–g, Rib cages from mutant mice and littermates were fixed in 4% paraformaldehyde, frozen in a cryostat, and processed for *in situ* hybridization as described in ref. 27, using a 634-nucleotide RNA probe from the AChR α subunit (accession number X03986). Sections were stained through the emulsion with toluidine blue, then photographed with dark-field (e, g) or bright-field (f) optics.

Because AChR clusters, conventionally used to identify synaptic sites, were absent in the mutant, we double-labelled sections with antibodies to SV2, a synaptic vesicle protein concentrated in nerve terminals²¹. In normal muscle, all four cytoskeletal components were concentrated in regions of the muscle membrane identified as postsynaptic because they were apposed by SV2-positive nerve terminals; utrophin was detectable only at synaptic sites, whereas the other three proteins were also present at lower levels extrasynaptically (Fig. 3*a, c*, and data not shown). In mutant muscle, in contrast, utrophin was undetectable (Fig. 3*b*), and α -syntrophin, α -dystroglycan and β -dystroglycan were all present in equal amounts in synaptic and extrasynaptic areas (Fig. 3*d*, and data not shown). These alterations suggest that rapsyn does not merely anchor AChRs to a preformed cytoskeleton, but is critical for organizations of the entire postsynaptic cytoskeletal complex.

Just as AChR aggregates overlie a specialized cytoskeleton, they are surmounted by a specialized basal lamina, which is structurally and functionally distinct from the extrasynaptic basal lamina with which it is continuous²². In aneural cultured myotubes, several components of synaptic basal lamina are co-localized with AChR/rapsyn coaggregates^{1,23}, raising the possibility that rapsyn is crucial for organization of synaptic basal lamina as well as the postsynaptic membrane. To test this idea, we stained mutant muscles with antibodies to three proteins that are concentrated in synaptic basal lamina *in vivo* and at AChR aggregates *in vitro*—acetylcholinesterase (AChE), s-laminin/laminin β 2 and agrin. All three proteins were concentrated at SV2-marked synaptic sites in the mutant, although levels of AChE were markedly reduced (Fig. 3*e–h* and data not shown). In both mutants and controls, AChE, derived largely from muscle, and laminin β 2, derived exclusively from muscle, were

restricted to synaptic sites, whereas agrin was present at lower levels in extrasynaptic regions, presumably reflecting its dual origin from nerve and muscle. We do not know what is responsible for synaptic localization of these basal lamina components in the mutant; one possibility is that they bind to the nerve terminal. However, LaRoche *et al.*²³ have reported that aggregates of laminin β 2 form on the surface of variant aneural myotubes that lack AChR/rapsyn aggregates. Together, our results and theirs suggest the existence of a postsynaptic scaffold that is distinct from AChRs and rapsyn.

Finally, based on strong evidence that target-derived retrograde signals influence the growth and differentiation of motor axons¹, we asked whether the failure of postsynaptic differentiation affects presynaptic differentiation. To assess the distribution of intramuscular axons, we stained whole muscles with antibodies to the synaptic vesicle proteins, synaptophysin or SV2. Synaptic vesicles are not restricted to the nerve terminal in neonates²⁴, so antibodies stained axonal bundles as well as nerve terminals. SV2 was more selectively associated with terminals than synaptophysin (Fig. 3*a'–h'*), so the latter was used to study axonal branching. In both mutants and controls, synaptophysin-positive axons left the main nerve trunk to branch in the central end-plate band (Fig. 4*a, c*). However, whereas wild-type axons formed short branches that ended in distinct terminal arbors, mutant axons generally formed long branches that ended without arborizing (Fig. 4*d*). Although the mutant terminals were simplified, their ultrastructure was qualitatively normal: synaptic vesicles were concentrated in the region of the terminal apposed to the muscle membrane and basal lamina was present in the synaptic cleft (Fig. 4*e, f*). However, junctional folds were occasionally present in the postsynaptic membrane of control but not mutant junctions. Moreover, numerous undifferentiated

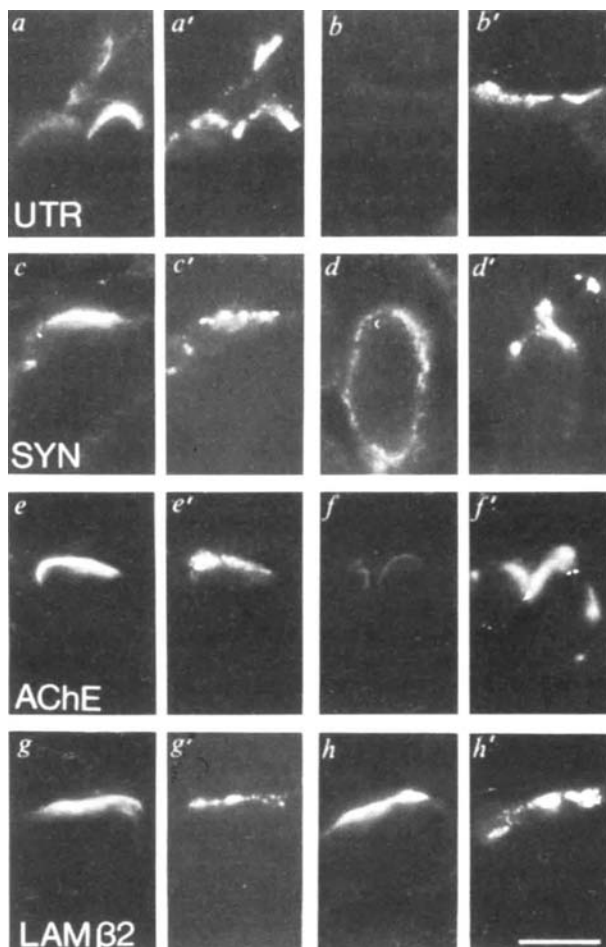


FIG. 3 Immunohistochemical analysis of neuromuscular junctions in normal (*a, c, e, g*) and rapsyn-deficient (*b, d, f, h*) mice. Sections of PO intercostal muscles were stained by an indirect immunofluorescence method with antibodies to utrophin (*a, b*), syntrophin (*c, d*), acetylcholinesterase (*e, f*) and laminin β 2 (*g, h*). Synaptic sites were visualized by co-staining the sections with antibodies to synaptic vesicle protein, SV2 (*a'–h'*). Muscle in *d* was overexposed to emphasize the uniform staining of synaptic and extrasynaptic portions of the muscle fibre surface. Scale bar is 10 μ m.

METHODS. Unfixed muscles were frozen in isopentane-cooled in liquid nitrogen, sectioned at 6 μ m in a cryostat and stained with antibodies as described²⁹. For double staining, we used second antibodies with contrasting fluorophores; fluorescein-conjugated goat-anti rabbit IgG and rhodamine-conjugated goat-anti mouse IgG. Anti- α -syntrophin FP2 antibody was a gift from K. P. Campbell and is described in ref. 16. This antibody was made against a fusion protein containing a C-terminal fragment of α -syntrophin, and appears to recognize only α 1 syntrophin. A rabbit polyclonal antiserum was to a 10-amino-acid C-terminal peptide from human utrophin (ref. 30, and W.-X. A. Guo and J.P.M., unpublished). Sources of antibodies are listed in refs 7, 16, 29 and 30.

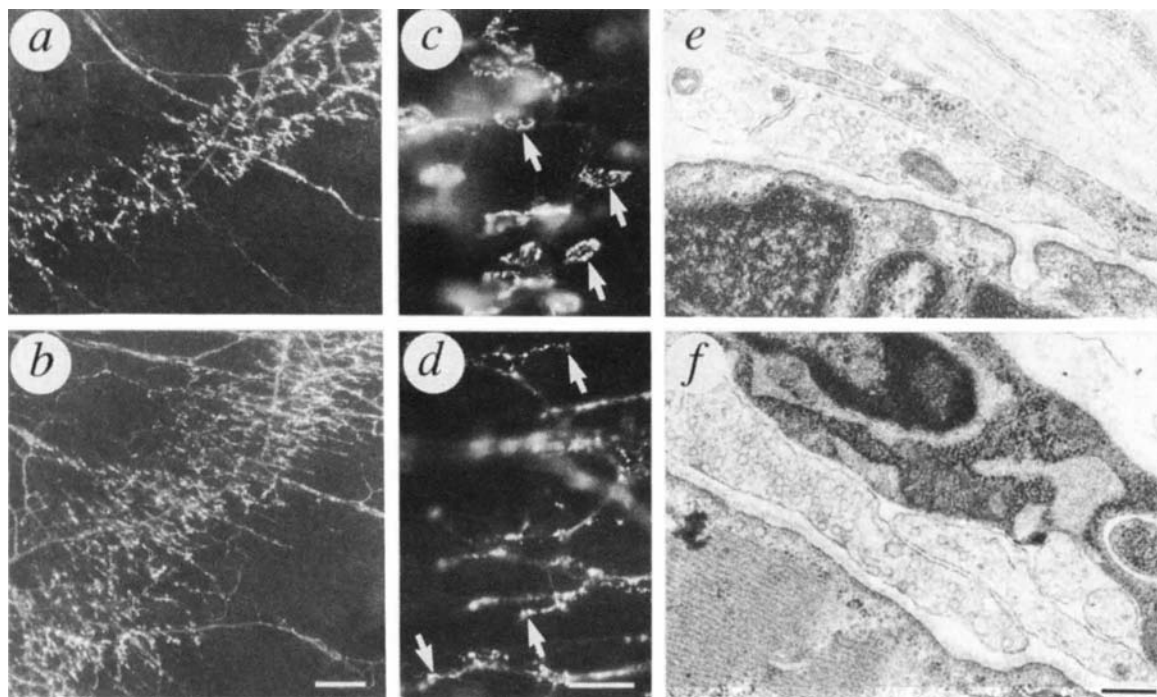


FIG. 4 Presynaptic differentiation in rapsyn-deficient mice and in littermate controls. *a–d*, Anti-synaptophysin staining in nerve terminals of PO gluteal muscles of normal (wild-type or heterozygote) and mutant mice. The extent of nerve branching was increased in mutants (*b*) compared to controls (*a*). Individual axons in control muscles terminated in distinct arbors (arrows in *c*), whereas mutant nerve terminals lacked arbors (arrows in *d*). *e* and *f*, Electron micrographs of neuromuscular junctions in normal (*e*) and mutant (*f*) PO intercostal muscles. Mutant and control terminals both bore concentrations of synaptic vesicles. Scale bar is 200 μ m in *b*, 10 μ m in *d* and 0.25 μ m in *f*.

axons ran between muscle fibres without forming specialized contacts in mutants but not controls, consistent with the light microscopic results (Fig. 4*a, b*). Thus, motor axons grew excessively and arborized poorly in the rapsyn-deficient mutant, but did form specialized presynaptic nerve terminals. Mechanisms linking rapsyn-deficiency to axonal abnormalities remain to be elucidated, but could include absence of rapsyn-anchored recognition molecules, diffuse release of sprouting factors normally released focally, and decreased synaptic activity resulting from the lowered density of AChRs in the mutant postsynaptic membrane.

In summary, we have provided genetic evidence that rapsyn is required for the aggregation of AChRs at the developing vertebrate neuromuscular junction. Several components of the AChR-associated cytoskeleton also fail to concentrate at mutant synaptic sites, indicating that rapsyn is critical for organization of the postsynaptic cytoskeleton. On the other hand, synaptic nuclei selectively transcribe AChR subunit genes in the mutant, demonstrating that local (presumably nerve-derived) factors use distinct intracellular signalling pathways to induce AChR synthesis and clustering. Moreover, several muscle-derived synaptic basal lamina components are concentrated at mutant synaptic sites, suggesting the existence of a second, rapsyn-independent synaptic scaffold. Finally, the presynaptic abnormalities we observed provide a starting point for seeking target-derived determinants of axonal arborization whose localization or expression is regulated by rapsyn. □

METHODS. *a–d*, Gluteal muscles were fixed in 2% paraformaldehyde in PBS, cleared with 0.1 M glycine in PBS, and permeabilized with 0.5% Triton X-100 in PBS with 4% BSA. Muscles were then incubated sequentially with rabbit antibodies to synaptophysin and fluorescein-goat anti-rabbit IgG. *e* and *f*, Muscles were fixed in 4% glutaraldehyde and 4% paraformaldehyde in phosphate buffer, washed and fixed in 1% OsO₄, dehydrated and embedded in resin. Sections were cut at 500–700 Å, stained with lead citrate and uranyl acetate and viewed in an electron microscope.

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Attention-generated apparent motion

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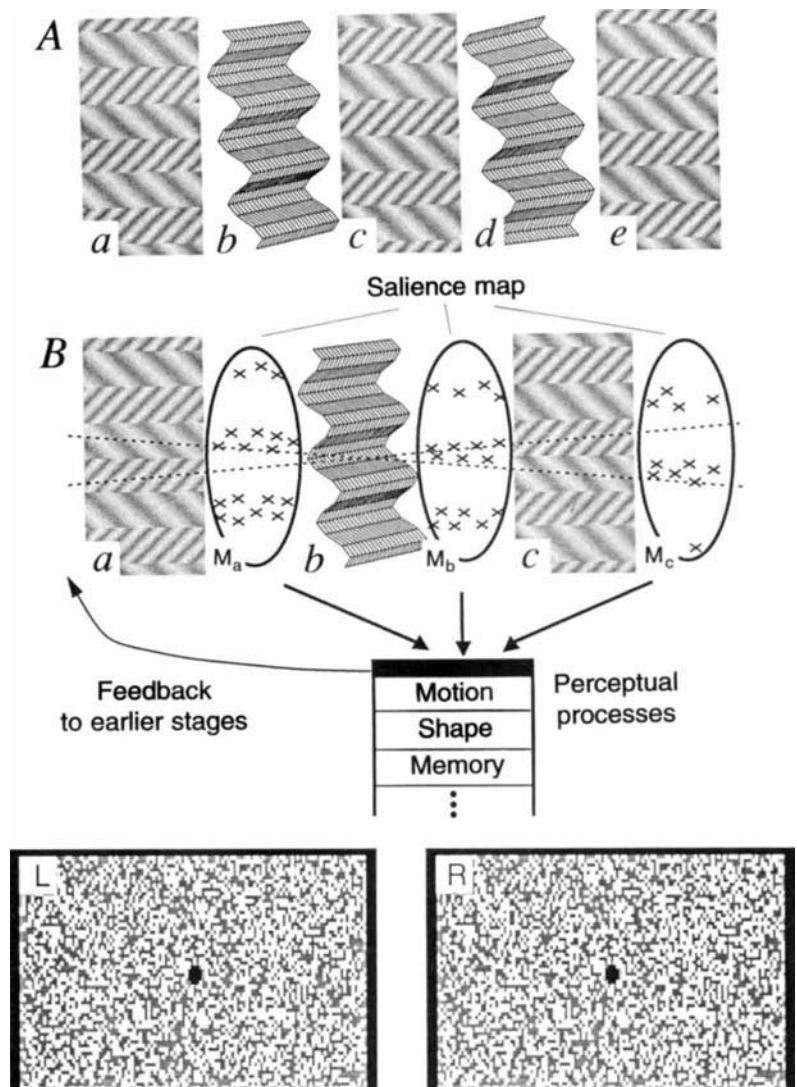
MOTION perception mechanisms have recently been divided into three categories¹. First-order mechanisms²⁻⁴ primarily extract motion from moving objects or features that differ from the background in luminance. Second-order mechanisms^{5,6} extract motion from moving properties, such as a moving area of flicker in which there is no difference in mean luminance between target and background. These first- and second-order motion mechanisms are primarily monocular. The existence of purely binocular, interocular and various other unusual kinds of apparent motion⁷⁻¹³ has promoted conjectures of a third-order mechanism^{1,14,15}, but there has been no clear suggestion as to the actual computations that such a mechanism might perform. Here we demonstrate 'alternating feature' stimuli that produce apparent motion only when the observer selectively attends to one of the embedded features in the display. The latent motion in the alternating feature stimuli is invisible to first- or second-order motion mechanisms, and the direction of apparent motion depends on the particular feature

attended. These findings suggest the mechanism of third-order motion: the locations of the most significant features are registered in a salience map, and motion is computed directly from this map.

How might a motion mechanism based on feature salience operate? At each moment in time, the x, y locations of the most significant features are marked (the salience map). For example, when figure-ground distinctions are meaningful, being marked leads to being labelled 'figure'. Motion is computed by standard algorithms²⁻⁴ from the spatio-temporal changes of the map. It is difficult to prove the existence of such a process because the same stimulus components that produce salient features usually also stimulate first- and second-order motion processes. The demonstration of feature-salience motion requires stimuli that are invisible to first- and second-order motion processes. The critical aspect is that only a feature's salience is marked from frame to frame, not the feature itself, so a feature-salience motion system transmits information only about the location, direction and speed of movement. Information about what is moving; that is, the features themselves, is carried by a pattern-processing system^{16,17}.

The key to demonstrating feature-salience motion is to use voluntary attention to influence which features are marked in ambiguous displays. It should not matter if the actual features being marked were to change from frame to frame, as long as their locations followed a constant motion trajectory, so we used alternating-feature displays. Frames made of one type of stimulus material were interleaved with frames made of another, entirely different, type of material¹³. All displays have

FIG. 1 Stimuli: the depth/texture alternating feature display. The top row (A) shows a sequence of five consecutive frames (a-e); each is displaced by 90 deg from the previous one. The depth stereograms (a, c, e) are indicated schematically; an actual stereogram is shown at the bottom, left (L) and right (R). The second row (B) shows the first three frames (a, b, c) and a feature-salience map (M_a, M_b, M_c) that might be associated with each frame. The most salient features of the depth frames are the near peaks (upper peaks in the panels) marked by crosses in the salience map (M_b). When a subject attends to the coarse grating, the coarse stripes are marked in the salience map (crosses in M_a and M_c). The direction of perceived motion follows the space-time trajectory of the crosses as indicated by the broken line from upper left to lower right. The opposite direction of motion has no support in M_a and M_c ; perceiving this upward motion would require attention to the fine stripes. Left (L) and right (R) eye images of a stereogram illustrating the bottom half of one frame of the depth stimulus. Viewing the left panel with the left eye and the right panel with the right eye shows 1.7 (of 3.7) cycles of the grating. The sinusoidal depth variation is quantized to three levels ($-1, 0, +1$ pixel disparity; each pixel subtends 1.5 min). The overall size of the stimulus as viewed by the subjects was 5.94×2.97 deg. The contrast of the texture gratings was 40%. The coarse stripes were sine waves, 2.5 cycles deg^{-1} , and the fine stripes were 5.0 cycles deg^{-1} .



the following properties. (1) Motion is invisible to the first- and second-order systems, and would be visible only to a feature-salience motion system. Perceiving motion would require combining salience information from the two different materials. (2) One stimulus feature perceptually dominates (is more salient) in one of the types of frames. (3) The to-be-attended features are entirely ambiguous (have equal salience) in the other type of frames. (4) Verbal instructions direct the subject to attend only to one of the two kinds of features in the feature-ambiguous frames. (5) Opposite motion directions are perceived depending on which feature is attended (marked as salient) in the feature-ambiguous frames.

Two different kinds of alternating-feature stimuli were created: depth/texture gratings (Fig. 1) and fullwave/halfwave gratings (Fig. 2). In the depth grating, the near-appearing peaks are naturally salient, and the two textures have equal salience. In the fullwave gratings, the high-contrast areas are naturally salient. In the halfwave gratings, after calibration for each subject, the areas of white and of black spots have equal salience.

In both stimulus types, the phase shift between successive frames is 90° (Figs 1 and 2), so successive even-numbered frames in the sequence are separated by 180°, as are successive odd-numbered frames. Motion between frames separated by 180° is

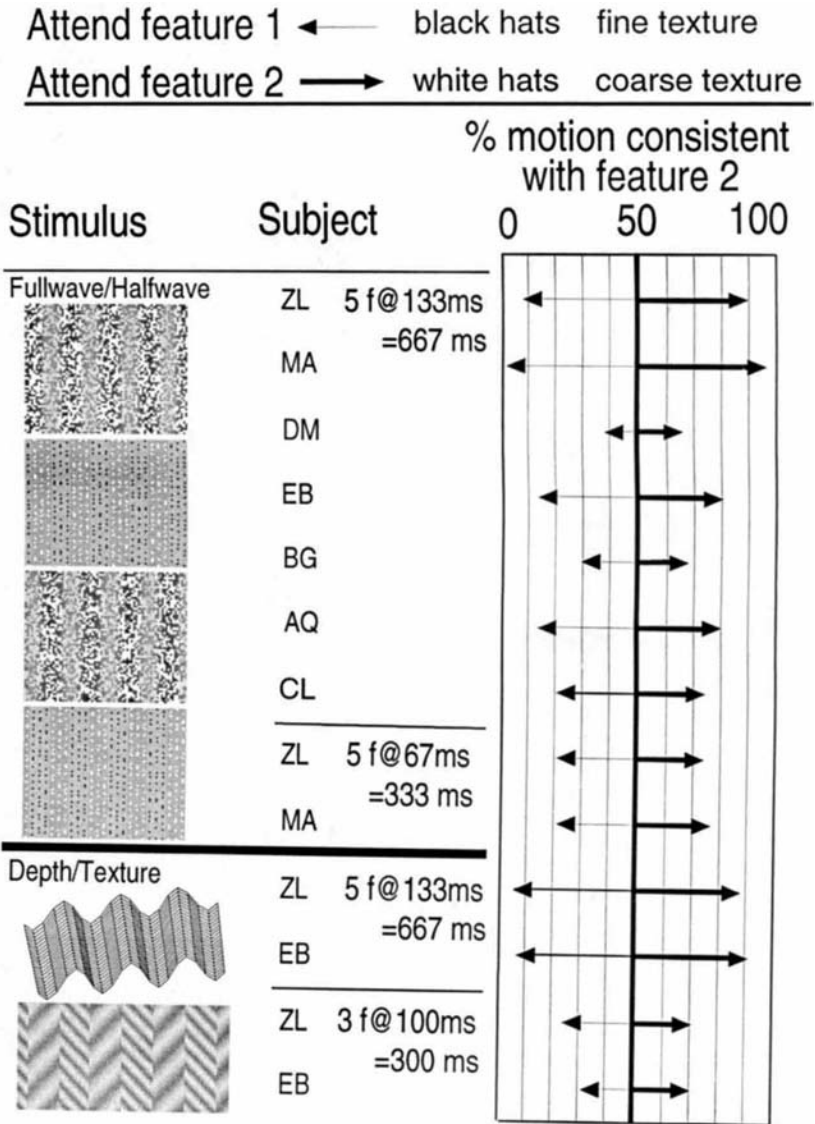
totally ambiguous. The perception of coherent motion would require combining information from even and odd frames, which are separated by 90°, the ideal separation for motion detection¹⁸. For each given alternating-feature motion display, the perceived motion direction depends on which stimulus feature is being attended in the feature-equivalent frames.

Some subjects immediately perceive motion in a direction consistent with the attentional instruction. Others do not. However, after several hundred trials, every subject who participated in the experiment perceived motion in a direction consistent with the attentional instruction. At this point, the instruction was reversed (attend to the previously unattended texture), and trials were continued until performance again stabilized. In no case did this take more than four blocks of 100 trials.

A small proportion of catch trials with unambiguous motion was used to determine whether subjects ever deliberately misreported the direction of apparent motion. No such reports occurred.

Very brief displays (total duration ≤ 333 ms) were constructed to exclude possible eye-movement¹⁹ mediation of perceived motion direction. Because the motion computation is the same with and without eye movements, eye-movement mediation would not logically alter any of the arguments of this paper.

FIG. 2 Procedures and results of the attention experiments. Subjects initiate a trial by pressing a key. A fixation point immediately appears in the centre of the display and remains on throughout the trial; 0.5 s later, a sequence of frames is presented. At the end of each trial, the subject indicates the direction of perceived motion. The notation 5f@133 ms = 667 ms indicates five frames, each of duration 133 ms, with a total display duration of 667 ms. Four frames of the fullwave/halfwave stimuli and two of the depth/texture stimuli are illustrated. Here the term fullwave²⁸ merely indicates alternating areas of high- and low-contrast random binary noise; halfwave indicates alternating areas of white dots on a dark-grey background and black dots on a light-grey background. Both frames have the same expected luminance everywhere and therefore do not stimulate the first-order (luminance) motion system. Before any attention instructions, the halfwave frames are calibrated individually for each subject to find a contrast ratio of white to black (between 1.00 and 1.18 for our subjects) for which the direction of perceived motion of the displays was completely random. This requires approximately 500 trials, 3 s per trial. This contrast ratio is used for all subsequent observations. Some subjects are then instructed to attend only to the white spots and others to attend only to the black spots. For example, the trajectory from high-contrast areas of fullwave frames (which are automatically salient) to white spots of halfwave frames (made salient by attention) produces perceived motion to the right. Only one type of attentional instruction is given within an experimental session. From trial to trial, the motion direction, the feature type of the starting frame, and the spatial phase of the starting frame are varied randomly. After a subject's performance has stabilized, the attention instructions are reversed, and the procedure is repeated. Two subjects, ZL and EB, were experienced psychophysical observers. The other subjects were naive student observers who were uninformed about the purpose of the experiments. For each subject the left-pointing arrow indicates data obtained from the last 200 trials with the instruction to attend to black spots (in fullwave/halfwave stimuli) and to fine textures (in depth/texture stimuli). A right-pointing arrow indicates data from attention to white spots (top) and to coarse texture (bottom). Initial calibration data, obtained under neutral attention conditions, fall within the black area occupied by the line under 50. Thus the tip-to-tip distance between oppositely pointed arrows indicates the



extent to which attention determines the apparent direction of apparent motion in these alternating feature displays.

Indeed, there is very little inclination to make eye movements: the display is 'much too quick'. The best strategy for seeing motion is to maintain very careful fixation and to peer through the display attempting to see the to-be-attended feature.

The results of the last 200 trials are shown in chronological order from top to bottom in Fig. 2. In the depth/texture condition, each subject consistently perceived motion in the direction that resulted from connecting the dominant depth feature (near-appearing peak) to the dominant texture feature (defined by the attentional instruction) in more than 90% of trials (chance is 50%). The same display was perceived as moving up or down, depending on which component texture the subject attended.

Similar results were obtained in the fullwave/halfwave condition. Subjects consistently perceived motion in the direction that resulted from connecting the dominant fullwave feature (high-contrast peak) to the attended halfwave feature (black or white spot as defined by the attentional instruction). The percentage of attention-conforming motion responses varied between subjects from 65–95%. All effects were highly significant statistically. The very brief displays (viewed only by the most consistently performing subjects) reduced attention-determined motion from over 90% to about 70%, which was still far above chance.

An error in calibration of the displays could have produced a consistent signal that might be detected by the first- or second-order motion system. Such an effect would appear as a bias to perceive motion consistent with one of the attentional instructions and not the other (a shortening or lengthening of the left-pointing arrows relative to the right-pointing arrows in Fig. 2). The near-perfect symmetry of the data verifies the absence of significant first- or second-order motion contamination.

The feature-saliency motion mechanism is affected by both top-down attentional processes and by automatic bottom-up processes^{20,21}. The large role of bottom-up inputs was demonstrated in some preliminary experiments. In one experiment, five frames were composed of a depth grating (near/far) alternating with the fullwave grating (contrast high/low). When subjects attend to high-contrast areas, they perceived motion in the direction consistent with movement from near depth to high contrast on 87–93% of trials. When they were asked to attend to low-contrast areas, no consistent motion direction was perceived. Attention could not overcome the large inherent asymmetries of feature saliency in the fullwave stimuli.

It is instructive to consider our results in relation to two quite different lines of research.

(1) When a colour and an achromatic grating are optically superimposed and moved in opposite directions, observers perceive only the motion of the achromatic grating. However, when the observers attentionally track a colour stripe, only the motion of the chromatic grating is perceived²². Cavanagh²² argued that this phenomenon is an instance of attentionally based motion perception, rather than merely an instance of attentionally selecting the output of one of two elementary motion computations, achromatic and chromatic. Just because the procedure requires conscious tracking to produce the perception of motion does not mean that tracking is the mechanism of motion perception. In our experiments, observers perceived attentionally determined motion even when stimuli were much too brief for conscious tracking. We assume that 'attentionally tracking' in Cavanagh's experiment²² involves the same saliency marking as does 'selectively attending' in ours, although tracking may also involve other mechanisms (such as efference copy²³). According to this interpretation, in both paradigms, third-order motion perception depends on an automatic stimulus computation, which in turn depends on the attentional state at the time of stimulus presentation.

(2) In perceptual search tasks, selective attention to features (such as red items among green items) seems to be mediated by directing attention to the location of the attended feature, and then processing the information at the location. The critical aspect of these results is that, in rapid sequences of dynamic

search displays, as in motion, selective attention is mediated via the locations of the attended features; attention does not directly exclude or admit items containing the feature^{24,25}.

All these phenomena are encompassed within the saliency map theory. A dynamic saliency map of the locations of the most salient stimulus features is determined jointly by stimulus strength (bottom-up) and by selective attention (top-down). Motion is computed directly and automatically from the saliency map, but the map can also be used to guide other processes, such as object recognition in attention-guided search and memory storage in location-cued recall tasks^{26,27}. □

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Hypertension in mice lacking the gene for endothelial nitric oxide synthase

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NITRIC oxide (NO), a potent vasodilator produced by endothelial cells, is thought to be the endothelium-dependent relaxing factor (EDRF) which mediates vascular relaxation in response to acetylcholine, bradykinin and substance P in many vascular beds^{1–6}. NO has been implicated in the regulation of blood pressure and regional blood flow^{4–6}, and also affects vascular smooth-muscle proliferation and inhibits platelet aggregation and leukocyte adhesion.

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Abnormalities in endothelial production of NO occur in atherosclerosis, diabetes and hypertension⁷. Pharmacological blockade of NO production with arginine analogues such as L-nitroarginine (L-NA) or L-N-arginine methyl ester affects multiple isoforms of nitric oxide synthase (NOS), and so cannot distinguish their physiological roles^{8,9}. To study the role of endothelial NOS (eNOS) in vascular function, we disrupted the gene encoding eNOS in mice. Endothelium-derived relaxing factor activity, as assayed by acetylcholine-induced relaxation, is absent, and the eNOS mutant mice are hypertensive. Thus eNOS mediates basal vasodilation. Responses to NOS blockade in the mutant mice suggest that non-endothelial isoforms of NOS may be involved in maintaining blood pressure.

Homozygous eNOS mutant mice, generated by homologous recombination (Fig. 1a, b), are viable, fertile and indistinguishable from wild-type and heterozygous littermates in appearance or routine behaviour. Immunoreactive eNOS protein is not present, as shown by western blot analysis of the brain, heart, lung and aorta (Fig. 1c). Calcium-dependent membrane-associated NOS enzymatic activity, assayed by ³H-arginine to citrulline conversion^{10,11}, was reduced from 12.8 pmol mg⁻¹ min⁻¹ in wild-type aorta to 0.5 pmol mg⁻¹ min⁻¹ in eNOS mutant aorta; the

residual activity is presumably due to neuronal NOS (nNOS) in neurons in the perivascular plexus.

Endothelium-derived relaxing factor is absent in eNOS mutant mice, as expected if eNOS is the source of EDRF. Aortic rings from wild-type mice manifest a dose-dependent relaxation to acetylcholine, as shown in Fig. 2a. Aortic rings from eNOS mutant animals show no relaxation to acetylcholine (Fig. 2c). Treatment of wild-type aortic rings with 10⁻⁴ M L-nitroarginine has no effect on vascular tone by itself, but blocks the relaxation in response to acetylcholine (Fig. 2b). Treatment of the eNOS mutant aortic rings with 10⁻⁴ M L-nitroarginine has no effect on vessel tone either by itself or on the response to acetylcholine. The maximum dilation of norepinephrine pre-contracted rings from eNOS mutant mice to sodium nitroprusside is no different from wild-type mice, indicating that vascular smooth-muscle responses are intact.

There are several interacting homeostatic regulators of blood pressure, including the renin-angiotensin system, the autonomic nervous system, and local mediators such as EDRF. We therefore examined basal blood pressure in the eNOS mutant mice to see if other homeostatic mechanisms would compensate for the absence of endothelial NO production, as they do for other

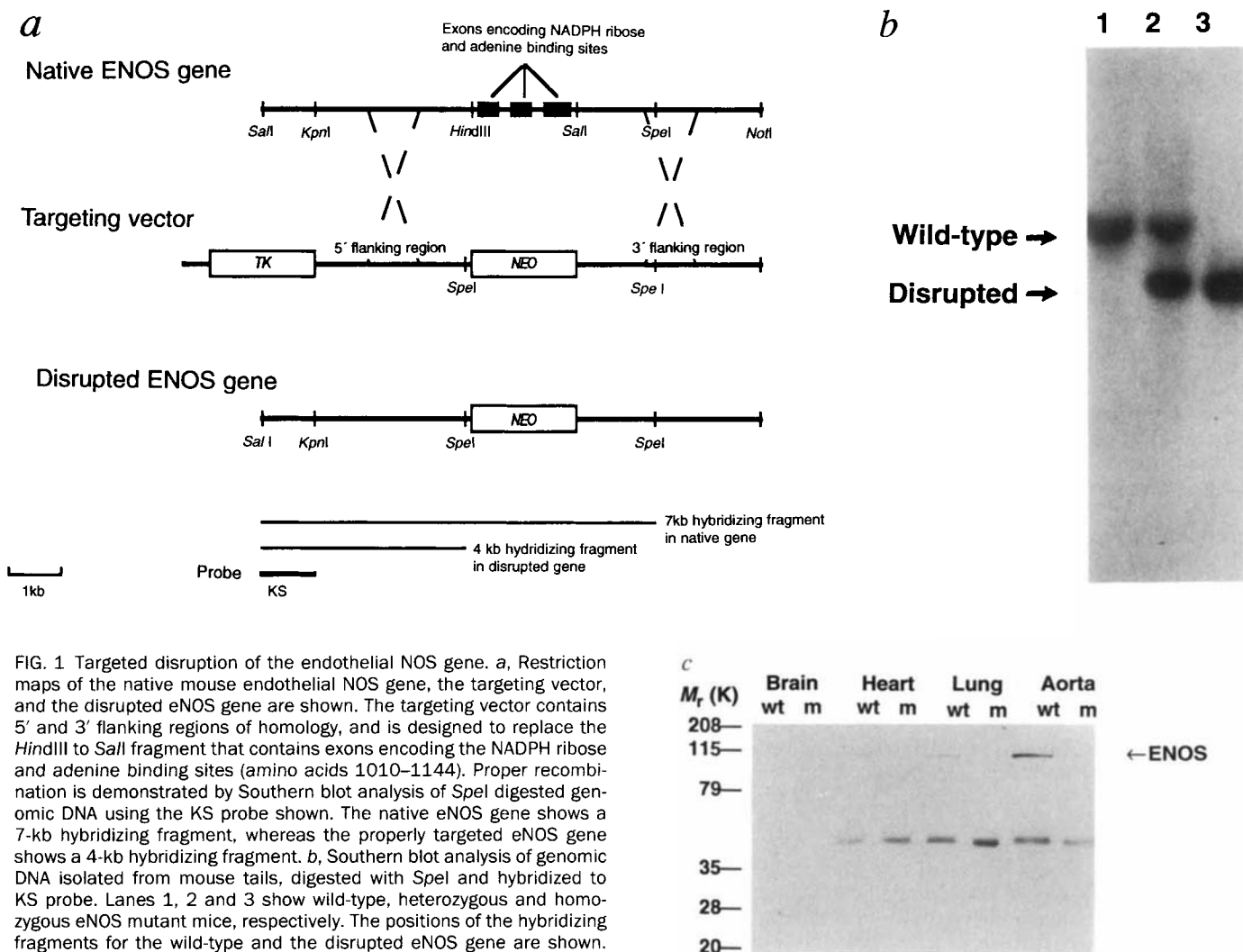


FIG. 1 Targeted disruption of the endothelial NOS gene. **a**, Restriction maps of the native mouse endothelial NOS gene, the targeting vector, and the disrupted eNOS gene are shown. The targeting vector contains 5' and 3' flanking regions of homology, and is designed to replace the *Hind*III to *Sall* fragment that contains exons encoding the NADPH ribose and adenine binding sites (amino acids 1010–1144). Proper recombination is demonstrated by Southern blot analysis of *Spe*I digested genomic DNA using the KS probe shown. The native eNOS gene shows a 7-kb hybridizing fragment, whereas the properly targeted eNOS gene shows a 4-kb hybridizing fragment. **b**, Southern blot analysis of genomic DNA isolated from mouse tails, digested with *Spe*I and hybridized to KS probe. Lanes 1, 2 and 3 show wild-type, heterozygous and homozygous eNOS mutant mice, respectively. The positions of the hybridizing fragments for the wild-type and the disrupted eNOS gene are shown. **c**, Western blot analysis of eNOS mutant mice. Protein extracts (10 µg) from the brain, heart, lung and aorta/vena cava (vessels) of wild-type (wt) and eNOS mutant animals (m) were electrophoresed through a 10% SDS polyacrylamide gel and transferred to nitrocellulose. The blot was incubated with mouse monoclonal antibody directed against endothelial NOS (Transduction Research Laboratories), and specific hybridization was localized using chemiluminescent alkaline phosphatase conjugated with anti-mouse antibody (ECL, Amersham). Hybridization of endothelial

NOS is present in the wild-type samples, but not in the eNOS mutant samples. The heart, lung and aorta samples show a band at relative molecular mass (*M_r*) 50K in both wild-type and eNOS mutant samples which represents mouse immunoglobulin heavy chains present in the tissue samples. The antibody used was directed against a peptide corresponding to amino acids 1030–1209, a region which overlaps with the region deleted in the eNOS mutant mice.

nitric oxide effects in eNOS mutant mice^{12,13}. The blood pressure of endothelial NOS mutant mice is significantly higher than littermates that are wild-type at the eNOS locus. Wild-type mice have a mean blood pressure of 81 mm Hg, whereas eNOS mutant mice have a mean blood pressure of 110 mm Hg (Table 1). Similar results are obtained with different methods of anaesthesia and in the awake state. Blood pressure is the same for the wild-type SV129 strain, wild-type C57B16 strain, and littermates of the eNOS mutant animals wild type at the eNOS locus.

L-NA and other NOS inhibitors cause a rise in blood pressure in many species, including rats, guinea pigs, rabbits, dogs¹⁴ and mice (Fig. 3). This effect is consistent with the predicted role for basal NO production in vasodilation, because inhibition of eNOS would lead to less basal vasodilation and result in hypertension. eNOS mutant mice show a decrease in blood pressure in response to L-NA (Fig. 3). This hypotensive effect of L-NA is prevented by L-arginine, and is not observed with D-nitroarginine, suggesting that pharmacological blockers may have effects in addition to NOS inhibition, or that non-endothelial NOS isoforms are involved in the maintenance of blood pressure. For

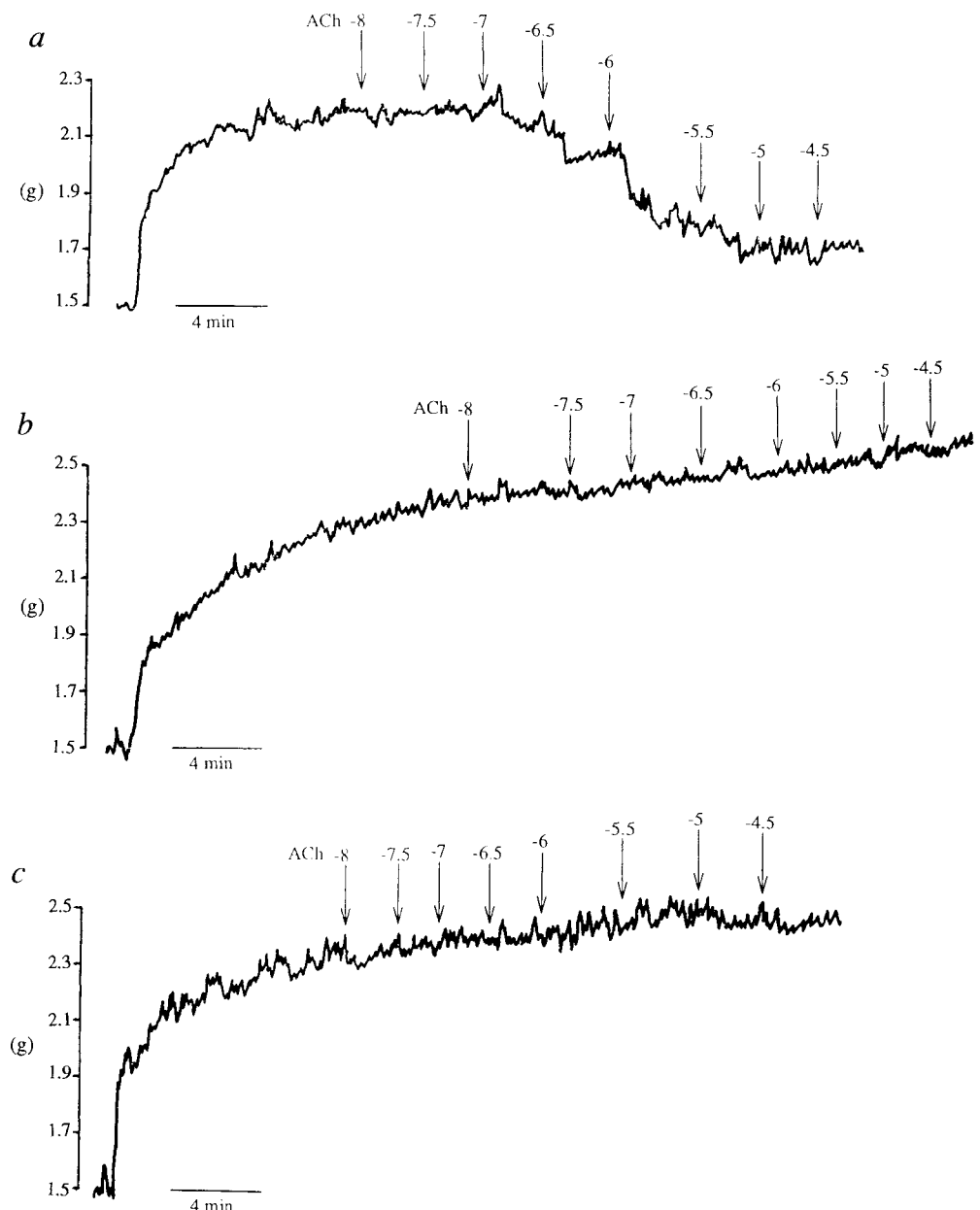
TABLE 1 Blood pressure of eNOS mutant and wild-type mice

	Wild-type mice			eNOS mutant mice		
	Urethane	Halothane	Awake	Urethane	Halothane	Awake
Mean BP (mmHg)	81	90	97	110*	109*	117*
s.d.	9	12	8	8	11	10
n	16	15	14	17	18	17

Mice were kept at normal temperature (37 °C), anaesthetized with urethane (1.5 mg per kg, intraperitoneal injection) or halothane inhalation, and ventilated using an SAR-830 mouse ventilator (CWE Instruments). Depth of anaesthesia was adjusted to keep the blood pressure of animals unresponsive to tail pinch with forceps. End-tidal CO₂ was monitored with a microcapnometer and kept constant by adjustment of respiratory parameters. The right femoral artery was cannulated using stretched PE-10 polyethylene tubing (Clay Adams) for mean arterial blood pressure recordings using a ETH-400 transducer and a MacLab data acquisition system (ADI Instruments). For awake measurements, the femoral artery catheter was placed under halothane anaesthesia and the wound was covered with 1% xylocaine ointment to diminish discomfort. Recordings were made within 1 h of the procedure. Data are expressed as means with s.d. Statistical evaluation was performed by t-test.

* $P < 0.01$ for eNOS mutant animals versus wild-type mice.

FIG. 2 Endothelium-dependent relaxation of aortic rings in response to acetylcholine. The thoracic aorta was dissected from wild-type and eNOS mutant mice and placed in physiological saline aerated with 95% O₂/5% CO₂. Segments (4 mm) were mounted on tungsten wires in conventional myographs and maintained at optimal tension in physiological saline for 1 h at 37 °C. The rings were precontracted with 10⁻⁷ M nor-epinephrine and exposed to increasing concentrations of acetylcholine (ACh) from 10⁻⁸ M to 3 × 10⁻⁵ M. *a*, Wild-type aortic segments respond to acetylcholine with dose-dependent relaxation. At 3 × 10⁻⁵ M, 60.3 ± 14.6% of preaddition tone is present. *b*, Treatment of the wild-type vessel rings with 10⁻⁴ M L-NA for 1 h abolished acetylcholine-induced relaxation. *c*, eNOS mutant aortic segments do not relax to acetylcholine, demonstrating that EDRF activity is absent from eNOS mutant mice. L-NA has no additional effect on eNOS vessel segments (data not shown). Segments (2–3) were collected from each mouse, and 5 mice were used from wild-type and eNOS mutant groups. Mean data from each animal were used. Representative tracings are shown. Acetylcholine concentrations are expressed as logM.



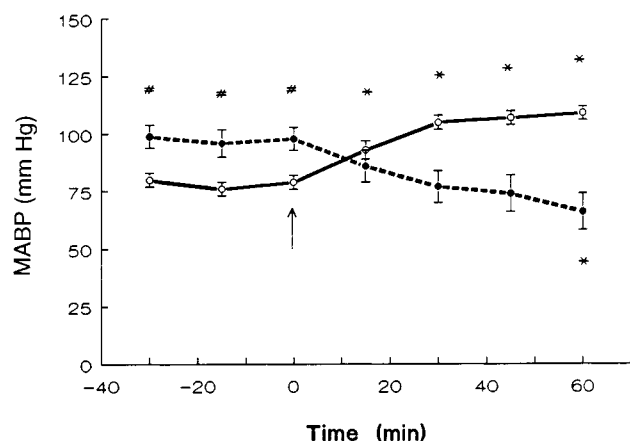


FIG. 3 Blood pressure responses to L-NA. Mean arterial blood pressure (MABP) of urethane-anaesthetized wild-type (solid line) and eNOS mutant mice (dotted line) were measured by femoral artery catheterization and recorded for 30 min of baseline before L-NA administration (arrows). At time 0, L-NA (12 mg per kg) was given intraperitoneally. Monitoring for 1 h shows that the blood pressure of wild-type mice rose from a baseline of 78 mm Hg to 109 mm Hg ($n=11$). The blood pressure of eNOS mutant mice dropped from a baseline of 98 mm Hg to 66 mm Hg ($n=5$). Each mouse in the wild-type group responded to L-NA with a rise in blood pressure, and each mouse in the eNOS mutant group responded with a drop in pressure. Mean arterial blood pressure differences between wild-type and eNOS mutant animals are statistically significant by *t*-test ($\#$, $P<0.01$). Differences between baseline blood pressures and following L-NA were also statistically significant ($*$, $P<0.05$ by ANOVA followed by Dunnett). These effects were prevented by L-arginine (200 mg per kg intraperitoneal) and not seen with D-nitroarginine (12 mg per kg); measurements in neuronal NOS mice showed similar results to wild-type mice (data not shown). The heart rate of eNOS mutant mice and wild-type mice did not differ, and L-NA had no effect on heart rate.

example, neuronal NOS (nNOS) is present both in vasomotor centres of the central nervous system and in perivascular nerves^{15–17}. However, effects of its blockade have suggested that it plays a vasodilatory role^{18–20}. Mice mutant in neuronal NOS have blood pressures similar to wild-type mice, but they have a tendency towards hypotension when exposed to anaesthesia²¹, which is consistent with a possible role for nNOS in maintaining blood pressure. Multiple roles for endothelial and non-endothelial NOS isoforms in vasodilation and vasoconstriction could explain the observed variability in maximal pressor effects of various NOS inhibitors^{22,23}.

Why are the eNOS mutant mice hypertensive? In other words, why don't other homeostatic systems compensate for absence of eNOS? The set point for systemic blood pressure is regulated through integration of cardiac, neuronal, humoral and vascular mechanisms. Perhaps the renin-angiotensin system and autonomic nervous system have evolved to serve primarily as a defence against hypotension, and diminution in their activity is a poor buffer against hypertension²⁴. Alternatively, eNOS may be involved in establishing the baroreceptor set point. Indeed, NO and NOS inhibitors affect baroreceptor responses^{25–27}. The question of whether subpopulations of humans suffering from hypertension have defects in eNOS expression awaits genetic analysis and the development of more selective inhibitors of the NOS isoforms. □

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Interaction of the erythropoietin and stem-cell-factor receptors

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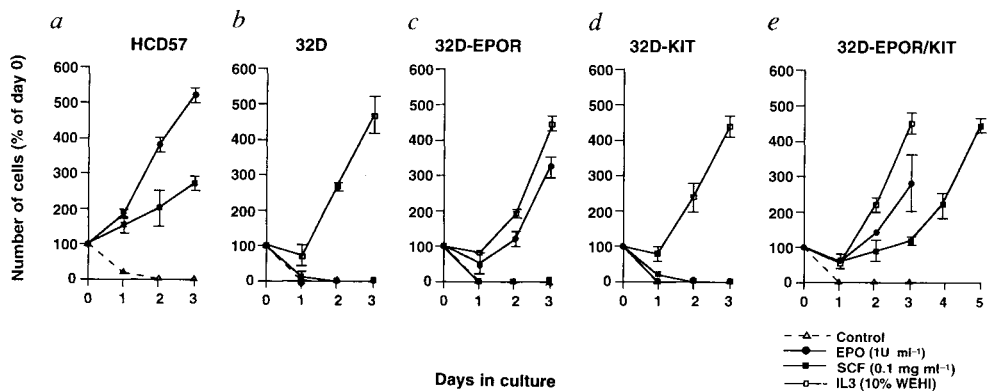
MUTATIONS in the KIT transmembrane protein-tyrosine kinase receptor¹ affect erythropoiesis, resulting in fewer committed late progenitors (colony-forming unit erythroid, CFU-E) in the fetal liver². As the survival and proliferation of CFU-Es depend absolutely on erythropoietin (EPO)³, these results suggest that CFU-Es cannot proliferate or mature further unless both the KIT and EPO receptor⁴ signalling pathways are functional. How KIT affects proliferation or differentiation of CFU-Es is not clear. Here we show that the KIT ligand SCF (for stem-cell factor) can replace EPO in supporting the growth and survival of HCD57 cells, an EPO-dependent erythroid-progenitor cell line expressing high levels of KIT⁵. SCF supports the proliferation of 32D cells⁶ that express KIT only if they also express the EPO receptor. In HCD57 cells, SCF rapidly induces tyrosine phosphorylation of the EPO receptor, and KIT physically associates with the extended box 2 region⁷ in the cytoplasmic domain of the EPO receptor. Our results indicate that KIT may activate the EPO receptor by tyrosine phosphorylation to induce further proliferation and maturation of CFU-Es.

HCD57 is an EPO-dependent erythroid progenitor cell line expressing high levels of KIT (see below). Unlike other cell lines in which SCF has little ability on its own to stimulate the growth of haematopoietic progenitors⁸, SCF can replace EPO in supporting the growth and survival of HCD57 cells (Fig. 1a). To determine whether the presence of the erythropoietin receptor (EPO-R) is essential for KIT function in erythroid cells, we used

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FIG. 1 EPO-R-mediated SCF-dependent cell proliferation.

METHODS. HCD57 cells were maintained in IMDM medium supplemented with 20% FCS and 1 U ml^{-1} recombinant human EPO (rhEPO; from Amgen). 32D and 32D-EPOR cells were cultured in RPMI1640 medium with 10% FCS and 5–10% conditioned medium from the WEHI-3B cell line as a source of IL-3. To generate populations of 32D and 32D-EPOR cells that also express KIT (32D-KIT and 32D-EPOR/KIT, respectively), cells were cocultivated for 2 days with an irradiated retrovirus-producing line expressing both wild-type *c-kit* and the neomycin-resistance(*neo*) gene⁹; cells were then selected in medium containing 0.8 mg ml^{-1} G418 for 10 days. For growth assays, cells were washed 3 times with IMDM or RPMI1640 medium and cultured for 18 h without EPO (for HCD57) or IL-3 (for 32D, 32D-EPOR, 32D-KIT and 32D-EPOR/KIT cells); they were then plated at 1×10^4 cells per ml in medium without growth factor (control), or supplemented with rhEPO (EPO



1 U ml^{-1} , or recombinant murine SCF (rmSCF; from the Genetics Institute, Cambridge, 100 ng ml^{-1}), or mIL-3 (IL-3 in 10% WEHI-3B conditioned medium). Viable cells were counted each day for 3 d, except for 32D-EPOR/KIT cells, which were counted for 5 d. At suboptimal concentrations of either SCF or EPO, EPO and SCF act synergistically in stimulating growth of HCD57 cells (data not shown).

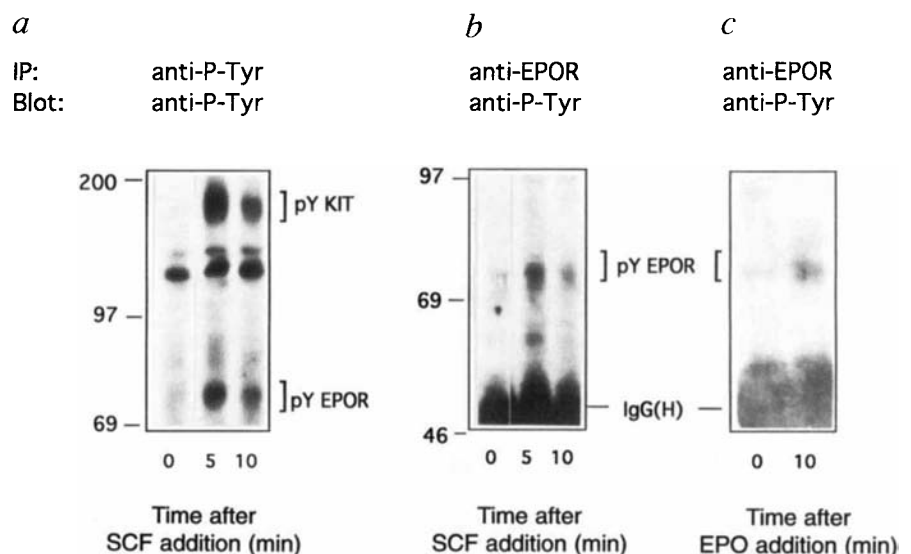
32D cells, an interleukin-3-dependent myeloid cell line that does not express KIT or EPO-R. 32D-EPOR cells, which express a transfected wild-type EPO-R, proliferate and survive in the presence of EPO alone, whereas parental 32D cells do not (Fig. 1b, c). Wild-type KIT was introduced into these two cell lines by infection with a recombinant retrovirus⁹. Pools of 32D-KIT and 32D-EPOR/KIT cells showed similar cell-surface expression of KIT, as judged by FACS analysis, and KIT in both cell lines became tyrosine-phosphorylated upon SCF stimulation (data not shown). Importantly, only 32D cells expressing both EPO-R and KIT could grow in response to SCF alone; 32D cells expressing KIT but not EPO-R could not (Fig. 1d, e). The effects of SCF on 32D-EPOR/KIT cells was not transient as it supported cell proliferation and survival even after 30 days of cultivation. The EPO-R and interleukin-3-receptor (IL3-R) are thought to use similar intracellular signalling molecules to sup-

port cell survival and proliferation, but only EPO-R, not IL-3-R, was able to collaborate with KIT in supporting SCF-dependent growth in 32D cells. Thus KIT is probably affecting EPO-R directly rather than downstream signalling molecules.

In HCD57 cells, activation of KIT by addition of SCF induced tyrosine phosphorylation of the EPO-R, as shown by immunoprecipitation of cell lysates with an anti-phosphotyrosine antibody (Fig. 2a) or by using an anti-EPO-R antibody¹⁰ (Fig. 2b) and western blotting with an anti-phosphotyrosine antibody. Tyrosine-phosphorylated EPO-R was also detected when HCD57 cells were stimulated by EPO (data not shown). We have done the same analyses on P815 cells^{11,12}, a murine mastocytoma cell line that expresses both EPO-R and KIT, and obtained similar results (data not shown).

FIG. 2 SCF stimulates tyrosine-phosphorylation of the EPO-R. Two major tyrosine-phosphorylated proteins were detected, one corresponding to the Golgi-processed form of KIT²⁰, and the other, with $M_r \sim 72\text{--}78\text{K}$, comigrating with tyrosine-phosphorylated EPO-R^{16,21} (a). The latter was tyrosine-phosphorylated EPO-R as it was also immunoprecipitated by an anti-EPO-R antibody (b). Maximum tyrosine phosphorylation occurred at 5 min after SCF addition and decreased thereafter. As an important negative control, no tyrosine-phosphorylated proteins could be precipitated by the anti-EPO-R antibody from 32D-EPOR cells after stimulation with SCF. However, EPO addition to these cells caused the expected transient tyrosine phosphorylation of the EPO-R (data not shown).

METHODS. Growth factor was removed from HCD57 cells by washing them 3 times with IMDM medium and then the cells were cultured in IMDM medium without serum and rhEPO for 4 h at 37°C . Cells were then incubated with $1,000 \text{ ng ml}^{-1}$ rmSCF (a, b) or 10 U ml^{-1} rhEPO (c) without serum for the indicated times, then collected by centrifugation and lysed in buffer containing 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.5% N-P40, 1 mM Na_3VO_4 , 10 mM NaF, 1 mM PMSF and 1% aprotinin. Extracts were precleared by incubation with protein-A agarose beads and then immunoprecipitated with an anti-phosphotyrosine antibody (a gift from J. L. Guan; a), an antibody against a GST fusion protein containing the extracellular domain of the EPO-R¹⁰ (b), or an antipeptide antibody specific for the C terminus of the EPO-R¹³ (c). The immunoprecipitates were



resolved by electrophoresis through a 7.5% polyacrylamide gel containing SDS, followed by western blot analysis using the anti-phosphotyrosine monoclonal antibody 4G10 ($1 \mu\text{g ml}^{-1}$; from UBI) and the enhanced chemiluminescence detection system (Amersham). Tyrosine-phosphorylated KIT (pY KIT), EPO-R (pY EPO-R), and the IgG heavy chain (IgG(H)) are indicated.

The rapid tyrosine-phosphorylation of the EPO-R in HCD57 cells after addition of SCF suggests that KIT may be physically associated with the EPO-R. As indicated by immunoprecipitation and western blot analysis (Fig. 3), only the slower-migrating Golgi-processed form of KIT could be immunoprecipitated with anti-EPO-R antibodies¹³ (Fig. 3, lanes 3 and 4), and only this form of KIT became tyrosine-phosphorylated when HCD57 cells were stimulated by SCF (Fig. 2a).

To determine the region of the EPO-R that associates with KIT, glutathione *S*-transferase (GST) fusion proteins were generated (Fig. 4a). As shown in Fig. 4b, only a fusion protein containing the cytosolic region of the EPO-R (GST-iEPO-R) could associate with KIT. More KIT proteins were bound to GST-iEPO-R protein (GST-iEPO-R(P)) that had been tyrosine-phosphorylated *in vivo* by Elk kinase^{14,15}, suggesting that KIT and EPO-R interaction depends, at least in part, upon a phosphotyrosine residue in the cytosolic domain of the EPO-R.

A set of fusion proteins containing truncations and point mutations in the cytoplasmic domain of the EPO-R was used to define further the domain mediating the association with KIT. As shown in Fig. 4a, c, GST-iEPO-R t374, a fusion protein containing amino acids 248–374, associated with KIT with an affinity comparable to that of unphosphorylated GST-iEPO-R; GST-iEPO-R t339, in contrast, was unable to bind to KIT. Thus, these truncation mutants defined 35 amino acids (339–374) in the cytoplasmic domain of the EPO-R as being necessary for association with KIT. GST-iEPO-R t374 bound much less KIT than did an *in vivo* phosphorylated fusion protein containing the entire cytoplasmic domain (GST-iEPO-R(P)), suggesting that tyrosine residues in the C-terminal 108 amino acids of the EPO-R are also important for KIT binding. To assess

this probability, we used GST fusion proteins containing tyrosine (Y)-to-phenylalanine (F) point mutations in four out of eight tyrosine residues in the cytoplasmic region of the EPO-R. The double mutation Y460/464F had no effect on association of the EPO-R with KIT; Y429/431F, however, bound significantly less KIT protein (Fig. 4c). To determine whether EPO-R could be a direct substrate of KIT kinase, we assayed kinase activity *in vitro*. Only GST-iEPO-R and GST-iEPO-R t374, but not GST-iEPO-R t339, could be phosphorylated by KIT immunoprecipated from HCD57 cells (data not shown).

In this study we have shown that signalling through the EPO-R is essential for function of the tyrosine-kinase receptor KIT in 32D cells, and that KIT can interact with and trigger tyrosine phosphorylation of the EPO-R. The EPO-R belongs to the cytokine-receptor superfamily, which is defined by the regions of similarity in their exoplasmic domains and which lack kinase- or phosphatase-related sequences in their cytosolic domains. Upon EPO addition, the EPO-R and JAK2 become tyrosine-phosphorylated^{16,17} and JAK2 binds to the box 1 and box 2 regions of the EPO-R (Fig. 4a); these segments of the EPO-R are essential for JAK2 activation, receptor tyrosine-phosphorylation, and mitogenesis^{7,18}. We have also shown that KIT binds, at least in part, to the 35-amino-acid region 339–374 that is part of the extended box 2 region; this segment of the EPO-R is critical for mitogenesis in FDC⁷ and Ba/F3-EPO-R cells (U.K., unpublished results) but not for activation of JAK2. This segment of the EPO-R, possibly because of its interaction with KIT, may transduce mitogenic or differentiation signals independently of JAK2 activation. It is possible that the KIT and JAK2 kinases phosphorylate, cooperatively or independently, different tyrosine residues in the cytoplasmic domain of the

FIG. 3 Co-immunoprecipitation of EPO-R with the Golgi-processed form of KIT. *M_r* markers are indicated on the left.

METHODS. HCD57 cells were maintained in 1 U ml⁻¹ rhEPO (lanes 1, 3, 4, 6) or 100 ng ml⁻¹ rmSCF (lane 7). 32D-EPO-R cells (lanes 2, 5) were cultured in RPMI1640 medium with 10% FCS and 10% WEHI-3B conditioned medium as a source of IL-3. Cells were washed several times with PBS and cell proteins were extracted in 0.5 ml of lysis buffer (150 mM NaCl, 10 mM Tris-HCl, pH 7.4, 1% Triton X100). Lysates were then mixed with 10 µl preimmune serum (lane 1), rabbit antipeptide antiserum¹³ against the C terminus (anti-C EPO-R, lanes 2 and 3) or N terminus of the EPO-R (anti-N EPO-R, lane 4), or 5 µl of anti-KIT antibody (lanes 5–7). After 1 h of incubation at 4 °C, 50 µl protein-A-agarose (Pierce) was added, and the samples were further incubated for 2 h at 4 °C with rotation. The protein A-agarose immunocomplexes were washed three times with lysis buffer and once with PBS, boiled in standard SDS-sample buffer (50 mM Tris-HCl, pH 6.8, 10% glycerol, 0.1% bromophenol blue; 2% β-mercaptoethanol) for 5 min, and subjected to electrophoresis through a 10% polyacrylamide gel containing SDS. Proteins were transferred to a nitrocellulose filter and immunoblotted with an anti-KIT antibody (1:1,000 dilution) in TBS containing 3% non-fat dry milk. After 1 h incubation at room temperature, the filter was washed three times in TBS containing 0.05% Tween-20, and reacted with ¹²⁵I-labelled protein A. After extensive washing in PBS, the filter was exposed with an intensifier screen to X-ray film at –80 °C for 5–14 days. Two forms of KIT proteins are seen in this experiment, the slower-migrating form of KIT with complex N-linked oligosaccharides and the faster species containing high-mannose N-linked oligosaccharides²⁰. A 145K protein, migrating in the same position as the form of KIT with complex N-linked oligosaccharides, was co-immunoprecipitated from HCD57 cells by both anti-C and anti-N EPO-R antibodies (lanes 3 and 4), whereas no similar proteins were immunoprecipitated from lysates of HCD57 cells by preimmune sera (lane 1). IgG(H) illustrates the position of the IgG heavy chain, a background protein in all of the samples. The amount of KIT co-immunoprecipitated with the EPO-R was quantified by using a Fuji Bioimager (FUJIX BAS 2000). Approximately 5% of the KIT protein in HCD57 cells (lanes 6 and 7) could be immunoprecipitated by anti-peptide antibodies¹³ specific for the N terminus (lane 4) or C terminus of the EPO-R (lane 3). As a negative control, no forms of KIT were immunoprecipitated by either anti-EPO-R or -KIT antisera from 32D-EPO-R cells that express the EPO-R but not c-KIT (lanes 2 and 5).

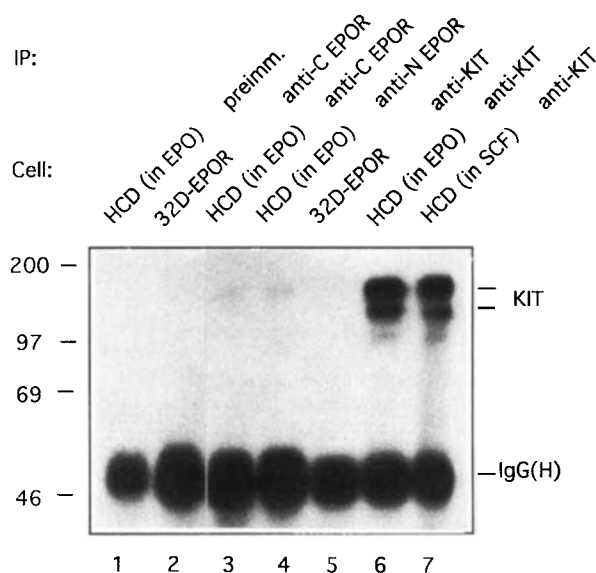
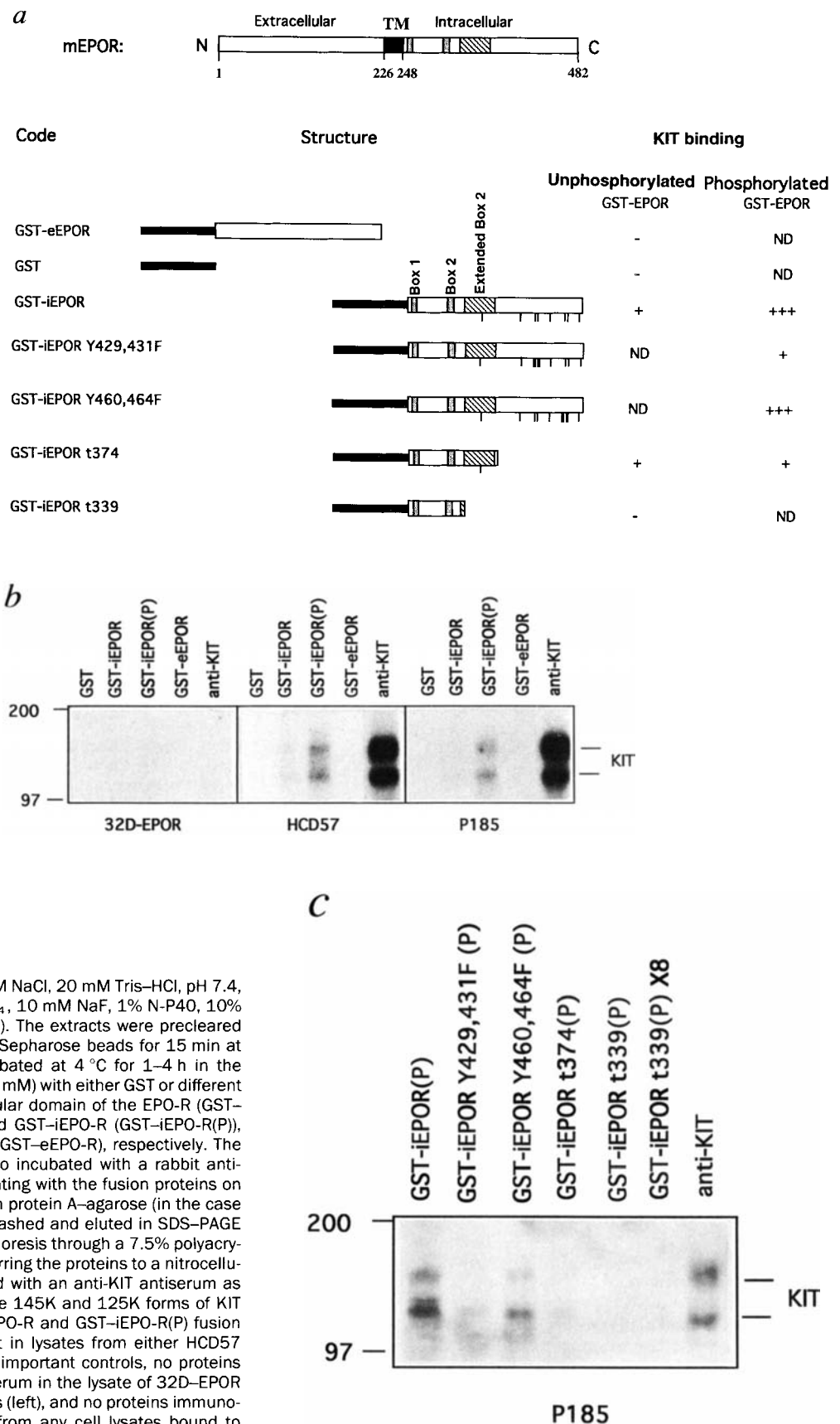


FIG. 4 KIT associates *in vitro* with fusion proteins containing the cytoplasmic domain of the EPO-R. **a**, A diagram of the fusion proteins used in this experiment. TM, transmembrane domain; filled boxes, box 1 and box 2 region; hatched box, extended box 2 region; small vertical bars, tyrosine residues that are potential sites of tyrosine phosphorylation in the cytoplasmic domain; bold vertical bars, Y to F point mutations. The DNA construct encoding GST-eEPO-R fusion protein was a gift from D. Hilton. To generate the series GST-iEPO-R fusion proteins, we first generated a DNA fragment corresponding to amino acids 248–339 of the EPO-R by PCR amplification. This fragment contained a *Bcl*I site at the 5' end and *Nco*I, *Hind*III, *Avr*II and *Eco*RI sites at the 3' end. The fragment was cloned into the *Bam*HI and *Eco*RI restriction sites of the pGEX-2T vector (Pharmacia), resulting in the construct GST-iEPO-R t339. The truncation t374 was then generated by inserting the *Sph*I-*Hind*III fragment of the cytoplasmic domain of the EPO-R into the *Sph*I and *Hind*III restriction sites of the GST-iEPO-R t339. The Y-to-F point mutations were introduced by ligating *Sph*I-*Avr*II fragments from the plasmids described by Klingmüller *et al.*¹⁰ to the *Sph*I and *Avr*II sites in the GST-iEPO-R t339. GST fusion proteins were prepared as described by Klingmüller *et al.*¹⁰. To tyrosine phosphorylate the fusion proteins, bacteria harbouring the gene fusion vectors were co-infected with a lysogenic λ phage encoding the cytoplasmic domain of the Elk tyrosine kinase¹⁴ and proteins were prepared as described in ref. 15. +, Detectable binding; +++, strong binding; ND, not done; –, no binding.

b, 32D-EPO-R, HCD57 or P815 cells were lysed in 0.3 ml lysis buffer (150 mM NaCl, 20 mM Tris-HCl, pH 7.4, 1 mM EDTA, 1 mM ZnCl₂, 1 mM Na₃VO₄, 10 mM NaF, 1% N-P40, 10% glycerol, 2 mM PMSF and 1% aprotinin). The extracts were precleared by mixing them with 30 μ l glutathione-Sepharose beads for 15 min at 4 °C. The cell extracts were then incubated at 4 °C for 1–4 h in the presence of BSA (1 mg ml⁻¹) and DTT (5 mM) with either GST or different fusion proteins containing the intracellular domain of the EPO-R (GST-iEPO-R), *in vivo* tyrosine-phosphorylated GST-iEPO-R (GST-iEPO-R(P)), the extracellular domain of the EPO-R (GST-eEPO-R), respectively. The same amounts of cell lysates were also incubated with a rabbit anti-KIT antibody (anti-KIT). Proteins associating with the fusion proteins on the Sepharose beads or complexed with protein A-agarose (in the case of anti-KIT immunoprecipitates) were washed and eluted in SDS-PAGE sample buffer and resolved by electrophoresis through a 7.5% polyacrylamide gel containing SDS. After transferring the proteins to a nitrocellulose filter, the filter was western blotted with an anti-KIT antiserum as for Fig. 3. The migration positions of the 145K and 125K forms of KIT are indicated at the right. Both GST-iEPO-R and GST-iEPO-R(P) fusion proteins bound to KIT proteins present in lysates from either HCD57 (middle panel) or P815 cells (right). As important controls, no proteins immunoreactive with the anti-KIT antiserum in the lysate of 32D-EPO-R cells bound to any EPO-R fusion proteins (left), and no proteins immunoreactive against the anti-KIT antisera from any cell lysates bound to the control GST-Sepharose. **c**, As for **b**, lysates from P815 cells were incubated with GST fusion proteins containing the truncated intracellular domain of the EPO-Rs (GST-iEPO-R t374 or GST-iEPO-R t339), or the intracellular part of the EPO-R carrying Y-to-F point mutations (GST-iEPO-R Y429, 431F, or GST-iEPO-R Y460, 464F), respectively. The



experiment in lane GST-iEPO-R t339 X8 is identical to that in GST-iEPO-R t339, except that 8-fold more fusion protein was used. Only a small fraction of the cell lysate was used for the anti-KIT antibody immunoprecipitation shown in the last lane.

EPO-R to provide docking sites for SH2 domains in other intracellular signal transduction proteins. SCF and KIT are also important for the survival, proliferation and migration of non-haematopoietic cells, such as primordial germ cells and melanoblasts, during mouse embryogenesis¹⁹. In these non-haematopoietic cells, KIT may phosphorylate and activate other lineage-specific receptors for cell proliferation and differentiation. □

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Distinct functions for the two importin subunits in nuclear protein import

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THE import of nuclear proteins proceeds through the nuclear pore complex and requires nuclear localization signals (NLSs)^{1,2}, energy^{3,4} and soluble factors⁵, namely importin- α (M_r 60K)^{6–12,28}, importin- β (90K)^{8–11,13} and Ran^{14,15}. Importin- α is primarily responsible for NLS recognition^{6–12,29} and is a member of a protein family that includes the essential yeast nuclear pore protein SRP1p (ref. 16). As the first event, the complex of importin- α and importin- β binds the import substrate in the cytosol^{8,9}. Here we show that this nuclear pore targeting complex initially docks as a single entity to the nuclear pore via importin- β . Then the energy-dependent, Ran-mediated translocation through the pore results in the accumulation of import substrate and importin- α in the nucleus. In contrast, importin- β accumulates at the nuclear envelope, but not in the nucleoplasm. Immunoelectron microscopy detects importin- β on both sides of the nuclear pore. This suggests that the nuclear pore targeting complex might move as a single entity from its initial docking site through the central part of the nuclear pore before it disassembles on the nucleoplasmic side.

To investigate the mechanism of nuclear protein import, we followed the import substrate, importin- α , and importin- β during protein import into nuclei of permeabilized⁵ HeLa cells.

Because the importin subunits differ slightly in size in different species, we propose calling them importin- α (60K subunit) and importin- β (the 90K subunit). Uptake of fluorescein-labelled nucleoplasmin depended on exogenously added importin complex (Fig. 1a), as reported previously^{7,8}. This also shows that efficient nuclear import can be achieved using only recombinant soluble factors, that is, Ran/TC4, importin- α and importin- β . Unlike Moore and Blobel¹⁷ and Paschal and Gerace¹⁸, we saw no stimulation of import efficiency by added recombinant pp15

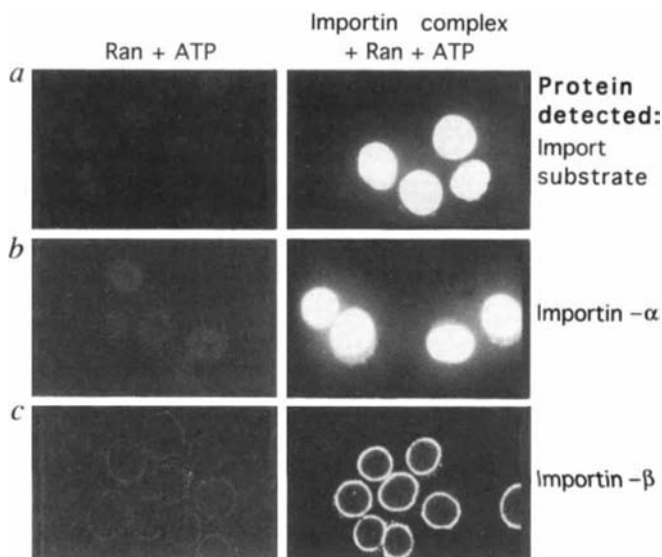


FIG. 1 The two subunits of importin become separated at the nuclear pore. Importin- α (60) enters the nucleus; importin- β (90) stays at the nuclear envelope. Import of nucleoplasmin into nuclei of permeabilized HeLa cells was studied in the absence (left panels) or presence (right panels) of importin- α and importin- β (importin complex). Fluorescein-labelled nucleoplasmin was detected by direct fluorescence as the import substrate in a. Unlabelled nucleoplasmin (1 mg ml^{-1}) was the import substrate in b and c, and importin- α (b) or importin- β (c) were detected by immunofluorescence. Ran and an energy-regenerating system were present in all reactions.

METHODS. Recombinant expression of Ran/TC4, *Xenopus* importin- α (untagged version) and nucleoplasmin, and preparation of permeabilized cells have been described elsewhere⁷. Human importin- β was expressed from a pQE60 vector (Qiagen) without a His-tag and was purified from the bacterial lysate by anion-exchange and affinity chromatography on immobilized importin- α . The assay contained 20 mM HEPES/KOH pH 7.5, 80 mM potassium acetate, 5 mM magnesium acetate, 250 mM sucrose, 100 μM ATP, 100 μM GDP, 50 μM GTP, 10 mM creatine phosphate, 50 μM 10^{-1} creatine kinase, 10 mg ml^{-1} BSA + 1 mg ml^{-1} nucleoplasmin core (to prevent nonspecific binding), 5 μM Ran (GDP form), and where indicated 500 nM importin- α and 200 nM importin- β . Reactions were started by addition of the NLS-recognition complex formed by importin- α , importin- β and nucleoplasmin and incubated for 30 min at room temperature. The reaction was stopped on ice by addition of 10 vols chilled buffer (20 mM HEPES, 80 mM potassium acetate, 5 mM magnesium acetate, 250 mM sucrose) followed by an equal volume of fixative (8% paraformaldehyde for direct fluorescence; 6% paraformaldehyde + 0.25% glutaraldehyde for immunofluorescence). Detection of import substrate was as described⁷. Immunofluorescence was carried out according to ref. 25. Fixation was followed by reduction with 1 mg ml^{-1} NaBH₄ in PBS, permeabilization with 0.1% Triton + 0.02% SDS in PBS and blocking with 5% BSA + 20% donkey normal serum. Anti-Ran/TC4 was raised against bacterially expressed Ran conjugated to keyhole limpet haemocyanin. Anti-importin- α ⁸ was against the recombinant *Xenopus* protein, anti-importin- β against recombinant human importin- β . All sera are mono-specific in blots. The anti-importin- α and - β sera were used after affinity purification on antigen columns. The anti-importin- α antibody was directly labelled with FLUOS (Boehringer); detection of importin- β was with fluorescein-isothiocyanate-labelled donkey anti-rabbit IgG secondary antibody (Amersham).

(data not shown); pp15 may remain in the cells during permeabilization in our conditions. Similarly, we observed a low concentration of endogenous importin- β at the nuclear envelopes of the permeabilized cells. This represents about 15% of the level seen with saturating amounts of exogenous importin- β (Fig. 1c), explaining the basal transport observed without exogenous importin- β ^{7,8,12}.

In Fig. 1b and c, unlabelled nucleoplasmin was used as the import substrate and the distribution of importin- α or - β was determined by immunofluorescence. Strikingly, when exogenous importin- α and - β were added together, importin- α accumulated in the nucleus whereas importin- β gave intense nuclear envelope staining and did not accumulate in the nucleus. Thus, if importin- β is released into the nucleosolic fraction at all, this occurs only transiently and its recycling must be rapid. Note that the assessment of importin- α and - β distribution during nuclear protein import shown in Fig. 1 does not rely only on the specificity of the antibodies used. Thus, as the patterns obtained depended on the addition of the importin complex, these results also represent a minus-antigen versus plus-antigen control for the specificity of immunofluorescence. Importin- α accumulated in the nuclei to a similar extent whether or not exogenous import substrate was added (data not shown), suggesting that importin- α can enter the nucleus with or without an import substrate.

If the entry of importin- α into the nucleus relates to faithful nuclear protein import, the accumulation of importin- α in the nucleoplasm should be Ran- and energy-dependent. To test this, import reactions with importin complex were carried out both in the absence and in the presence of Ran and energy (Fig. 2). Import substrate, importin- α , importin- β and Ran were detected by direct fluorescence or by immunofluorescence. In the presence of importin complex but without Ran/TC4 or energy, import intermediates accumulate at the nuclear envelope^{8,14}. Full transport occurs only with Ran and energy. Importin- α behaved in the same way as the import substrate (Fig. 2a); it accumulated

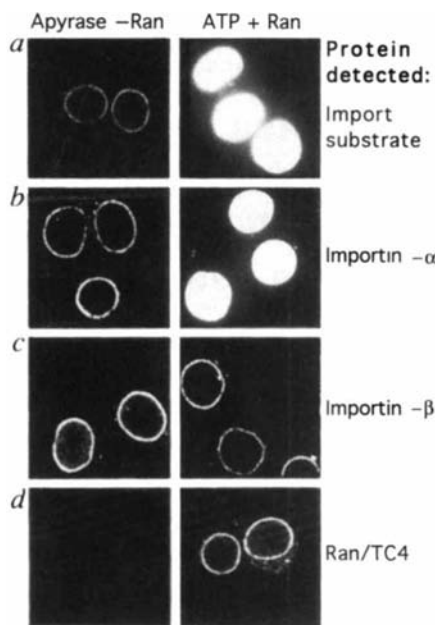


FIG. 2 Effect of Ran and ATP on the distribution of import substrate (a), importin- α (b) and importin- β (c). Ran allows the import substrate and importin- α to enter the nucleus, leaving importin- β at the nuclear envelope. Ran also binds to the nuclear envelope (d). The import reaction in the presence of importin- α and importin- β and the detection of the indicated proteins are described in Fig. 1 legend. Additions were as indicated: apyrase $0.1 \text{ U } \mu\text{l}^{-1}$; 'ATP' indicates $100 \text{ } \mu\text{M}$ ATP + 10 mM creatine phosphate + $50 \text{ } \mu\text{g ml}^{-1}$ creatine kinase, $50 \text{ } \mu\text{M}$ GTP; Ran (GDP form) was at $5 \text{ } \mu\text{M}$.

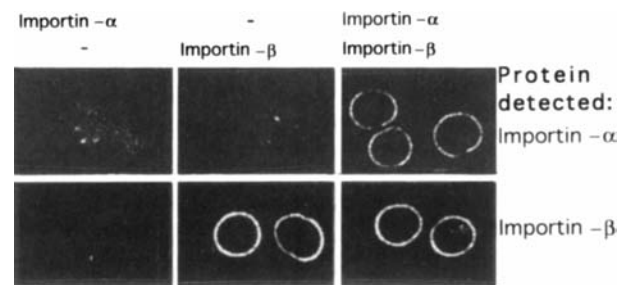


FIG. 3 Importin- β mediates the primary docking of the NLS-recognition complex to the nuclear envelope. Importin- α alone does not dock, whereas importin- β or the combination of the two subunits do dock. Nuclei were incubated with 500 nM importin- α and/or 200 nM importin- β as indicated. Bound importin- α and importin- β were detected by immunofluorescence as described for Figs 1 and 2. The reaction was carried out without Ran and ATP, with apyrase and in the presence of 1 mg ml^{-1} unlabelled nucleoplasmin as an import substrate.

at the nuclear envelope in the absence of Ran and ATP and entered the nucleus under conditions of full transport (Fig. 2b). Thus, these properties of importin- α substantiate Adam and Gerace's model of a shuttling nuclear import receptor¹⁹. Importin- β did not accumulate in the nucleus and was found at the nuclear envelope whether or not Ran and ATP were present (Fig. 2c). Exogenously added Ran was predominantly detected at the nuclear envelope (Fig. 2d), consistent with its central role in transport through the pore^{14,15}. The same antibody detects Ran as a nuclear antigen in intact cells (data not shown), as reported elsewhere^{20,21}. The apparent absence of Ran from the nucleoplasm under these conditions may be because its small size allows it to escape from the nucleus *in vitro* before fixation, just as it is lost during preparation of permeabilized cells (Fig. 2d, left panel; data not shown; and ref. 22), explaining why import is fully dependent on exogenously added Ran.

To determine which importin subunit contains the binding domain for the initial docking to the pore, we incubated nuclei with each subunit alone or with both together and assayed the binding of importin- α and - β to the nuclear envelope by immunofluorescence. Binding of importin- α was greatly enhanced by, if not fully dependent on, importin- β (Fig. 3). Importin- β , however, bound to the nuclear pores at similar levels whether or not importin- α was present. Thus, the initial docking of the NLS-recognition complex to the nuclear pore seems to be via importin- β .

At which stage of translocation through the nuclear pore does the NLS-recognition complex dissociate? At one extreme, importin- β might remain at its initial docking site while the complex between importin- α and import substrate translocates. At the other extreme, the NLS-recognition complex would move as a single entity through the pore, with the release of importin- α and the substrate into the nucleoplasm from importin- β being the last step. To investigate where the complex disassembles, we used immunoelectron microscopy on rat liver cryosections to find the furthest point at which importin- β could still be detected (Fig. 4). The highest concentration of gold was found along the nuclear envelope. The label was most prominent at the cytoplasmic side of the nuclear pore up to 50 nm from its centre. This probably corresponds to the cytoplasmic fibres at which the import substrate docks initially⁴. A smaller but significant proportion of gold labelled the central part of the pore. On the nucleoplasmic side of the pore significant gold was found within 50 nm of the pore, but labelling further inside the nucleus was close to background. In conclusion, it seems that importin- β moves over a distance of at least 100 nm from its initial cytoplasmic docking site through the central part of the nuclear pore complex to the nucleoplasmic side. This is consistent with the assumption that the complex of import substrate, importin- α

and β moves as a single entity through the nuclear pore complex, and suggests that the disassembly of the nuclear pore targeting complex is a late event or even the last event in translocation.

Within the NLS-recognition complex the β -subunit of importin seems to make direct contact with the nuclear pore complex. This suggests that the initial force driving the import substrate into the nucleus might be generated between importin- β and structures of the nuclear pore. The α -subunit might not only mark those proteins destined for the nucleus, but also prevent the highly charged NLS from nonspecific interactions. This would explain why importin- α accompanies karyophilic proteins from the sites of their synthesis through the pores to the sites of their function. Proteins shuttling between nucleus and cytoplasm²³ might also exit from the nucleus bound to importin- α . However, those proteins also clearly accumulate in the nucleus, so the discharge of the NLS must precede the re-export of importin- α into the cytoplasm. This could be achieved in three

ways. The disassembly of the importin complex in the nucleus could weaken the binding of the NLS to importin- α , facilitating its release; alternatively release might be mediated by association of importin- α with other nuclear factors, or by a covalent modification such as phosphorylation by a homologue of the recently discovered SRP1p kinase²⁴. These possibilities are being investigated. □

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Inhibition of the *Caenorhabditis elegans* cell-death protease CED-3 by a CED-3 cleavage site in baculovirus p35 protein

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THE baculovirus protein p35 inhibits programmed cell death in such diverse animals as insects, nematodes and mammals^{1–5}. Here we show that p35 protein is a substrate for and inhibitor of the *Caenorhabditis elegans* cell-death protease CED-3 (refs 6, 7) and a substrate for four CED-3-like vertebrate cysteine protease activities implicated in apoptosis in mammals^{7–14}. A p35 mutation that greatly reduced p35 activity *in vitro* as a CED-3 substrate and inhibitor abolished p35 activity *in vivo* in protecting against cell death in *C. elegans*. Introduction of the CED-3 cleavage site in p35 into the cowpox virus protein crmA, which inhibits mammalian apoptosis^{14–19} but not programmed cell death in *C. elegans*, caused crmA to block CED-3-mediated cell death. These observations suggest that p35 may prevent programmed cell death in *C. elegans* and other species by acting as a competitive inhibitor of cysteine proteases.

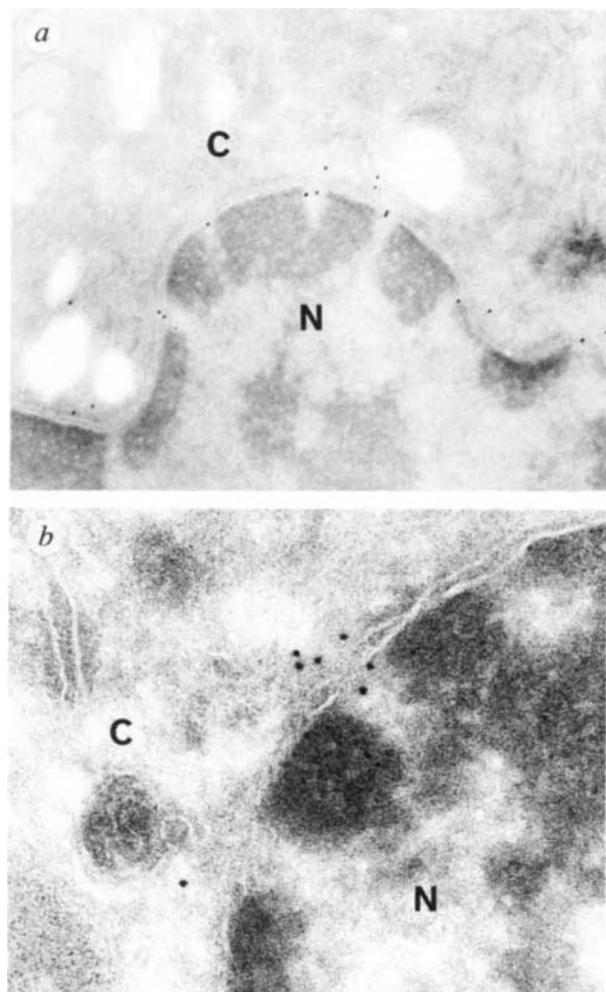
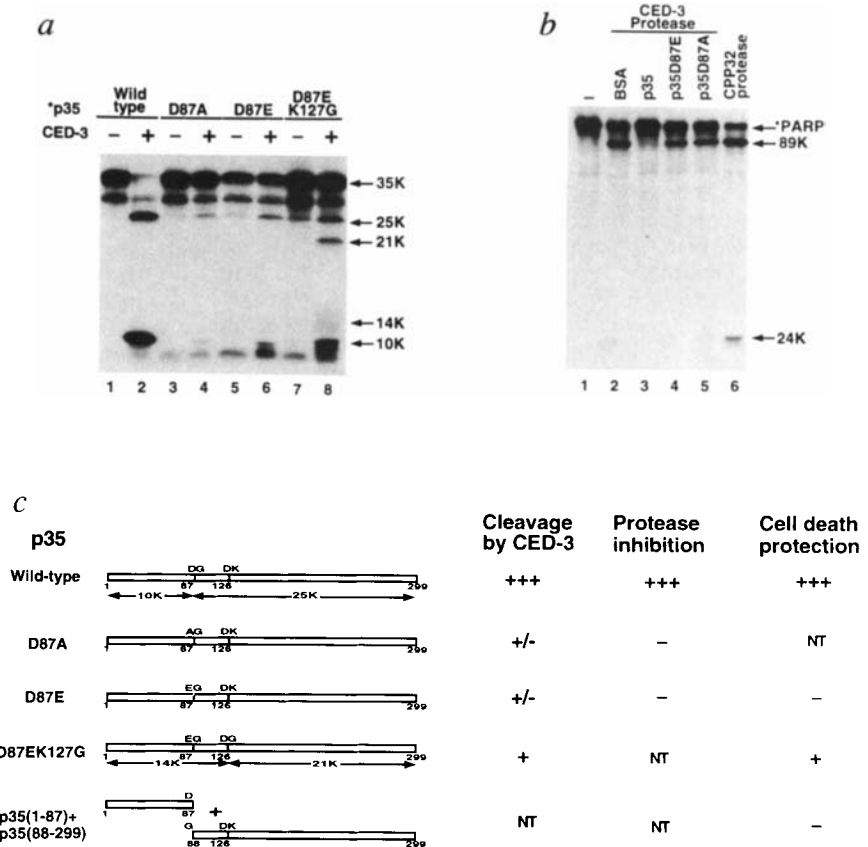


FIG. 4 Localization of importin- β at the nuclear pore by immunogold electron microscopy. The antibody in *a* was raised against recombinant importin- β and the antibody in *b* against an internal peptide⁸. N, nucleus—note nuclear pores adjoin pale euchromatin; C, cytoplasm. Both antibodies were affinity-purified and are monospecific on western blots of total tissue. Gold size, 10 nm.

METHODS. Rat liver was fixed for 1 h in 0.1% glutaraldehyde and 4% paraformaldehyde, 0.1 M sodium phosphate pH 7.4, 0.18 M sucrose. Pieces of fixed tissue were infused according to ref. 26 with 1.8 M sucrose and 20% polyvinylpyrrolidone (*M_w* 10,000), and cyrosectioned. Immunolabelling and detection with protein A–gold was according to ref. 27.

FIG. 1 Baculovirus p35 protein is a substrate for and inhibitor of the CED-3 protease. **a**, ^{35}S -methionine-labelled p35 protein (*p35) was incubated with (lanes 2, 4, 6 and 8) or without (lanes 1, 3, 5 and 7) 10 ng purified CED-3 protease for 10 min at 30 °C. **b**, ^{35}S -methionine-labelled PARP (*PARP) was incubated with buffer (lane 1), with 2 ng purified CED-3 protease in the presence of 200 ng of the purified proteins indicated (lanes 2–5) or with 2 ng purified CPP32 protease (lane 6) for 20 min at 30 °C. **c**, Summary of p35 *in vitro* and *in vivo* activities. NT, not tested. The cleavage of p35 proteins by CED-3 protease, the protease inhibition activity, and the protection against cell death (see Table 1) for different p35 proteins are scored as strong (+++), intermediate (+ or +/-) or no activity (-). The bands seen below the full-length p35 band in lanes 1, 3, 5 and 7 could be internal initiation products from methionines 35, 45, 46 and 85 of the p35 protein²¹.

METHODS. We excised from plasmid pPRM-35KORF²¹ an *Eco*RI fragment that contains a full-length p35 cDNA and cloned this fragment into the *Eco*RI site of pBluescript KS(+) (Stratagene)²⁸. Using the resulting plasmid pDX1, we constructed p35 mutations by *in vitro* mutagenesis²⁹. We synthesized and labelled with ^{35}S -methionine p35 proteins and PARP using the TNT coupled reticulocyte lysate system (Promega) and pDX1, its derivatives, and a plasmid that contains a full-length PARP cDNA¹⁴, as DNA templates. We incubated 1 μl of the translated reticulocyte lysate with 1 μl purified CED-3 or CPP32 protease (D.X. and H.R.H., unpublished results) and 2 μl ICE buffer²⁷. The reactions were analysed by 15% SDS-polyacrylamide gel electrophoresis (SDS-PAGE)²⁸. The gel was dried and subjected to autoradiography. Recombinant p35 proteins were tagged with the octapeptide FLAG (DYKDDDDK) and purified from *Escherichia coli* as described³⁰. For microsequencing, the *Eco*RI fragment from pPRM-35KORF was cloned into GST expression vector pGEX-1 λ T (Pharmacia) at the *Eco*RI site. The



GST-p35 fusion protein was purified as described²⁰. The purified fusion protein (10 μg) was digested with 300 ng purified CED-3 protease and then resolved by 12.5% SDS-PAGE. Microsequencing analysis was performed as described previously²³.

That p35 inhibits programmed cell death in a diverse range of animals suggests that it functions by interacting with an evolutionarily conserved component of a pathway for cell death. We examined the interaction of p35 with one such conserved component, the cell-death effector represented by the mammalian cysteine protease interleukin-1 β -converting enzyme (ICE)^{8,10} and the *C. elegans* protein CED-3 (refs 6, 7), which, like the pro-form of ICE, can autocleave to generate a cysteine protease (D.X. and H.R.H., unpublished results). We found that ^{35}S -methionine-labelled p35 protein synthesized *in vitro* was cleaved by purified CED-3 protease to generate two products of relative molecular masses (M_r) 10K and 25K (Fig. 1a, lanes 1 and 2). A fusion protein consisting of 26K glutathione *S*-transferase (GST)²⁰ fused at its carboxy terminus to p35 was cleaved similarly by CED-3 protease to yield two products of 36K and 25K, suggesting that the 10K product seen after digestion of p35 was located N-terminal to the 25K product (data not shown). We determined the N-terminal sequence of the 25K product by microsequencing (see legend to Fig. 1). The sequence (GFHDSI) indicated that the CED-3 protease cleaved p35 between amino acid residues aspartate 87 and glycine 88 (ref. 21). This cleavage site is similar to the known cleavage sites of substrates of ICE, which are between an aspartate and a small amino acid such as glycine and alanine^{8,9,22}.

We found that human poly(ADP-ribose) polymerase (PARP) was cleaved by the CED-3 protease to generate products indistinguishable in size from those generated by the CPP32/Yama protease, another member of the CED-3/ICE family^{11,14} (Fig. 1b, lanes 1, 2 and 6). PARP cleavage by CED-3 was inhibited by recombinant p35 protein (Fig. 1b, lane 3). Specifically, puri-

fied p35 protein when present in excess of the CED-3 protein (molar ratio approximately 100:1) completely prevented the generation of the 24K and 89K PARP cleavage products^{14,23}. By contrast, similar amounts of a control protein (bovine serum albumin) or of either of two purified mutant p35 proteins—with missense mutations at the aspartate 87 CED-3 cleavage site and greatly reduced in their activities as CED-3 substrates (Fig. 1a, lanes 3–6, c)—did not inhibit the cleavage of PARP by CED-3 (Fig. 1b, lanes 2, 4 and 5). Thus p35 inhibited CED-3 protease activity *in vitro*, and this inhibition required a functional CED-3 cleavage site in p35.

To determine if the presence of this CED-3 cleavage site is also important for p35 function in *C. elegans*, we used germline transformation to generate transgenic nematodes expressing either the wild-type or mutant p35 proteins under the control of the *C. elegans* heat-shock promoters *hsp16-2* and *hsp16-41* (ref. 24). As reported previously³, wild-type p35 protects against programmed cell death in *C. elegans*: we observed an average of 8.8 extra or 'undead' cells in the anterior pharynx of these transgenic worms after heat-shock treatment (Table 1). For comparison, *ced-3* mutants completely defective in cell death have an average of about 14 extra cells in this region²⁵. By contrast, no extra cells were seen in animals carrying heat-shock vectors alone or in p35D87E transgenic worms, suggesting that this mutant p35 protein lacked the ability to protect cells from programmed cell death.

To test if the protective function of p35 is conferred by its 10K and/or 25K cleavage products, we coexpressed these proteins in *C. elegans* under the control of the nematode heat-shock promoters. No protection was observed after heat-shock treatment

(Table 1), suggesting that these p35-derived proteins do not prevent programmed cell death when coexpressed as two separate proteins.

We introduced a second mutation, causing the replacement of lysine 127 with glycine, into the gene encoding the inactive D87E p35 protein. This amino acid change one residue after aspartate 126 generated a potential CED-3 cleavage site, aspartate-glycine. This doubly mutant p35 protein was cleaved by CED-3, albeit inefficiently, generating two new products of M_r 14K and 21K (Fig. 1a, lanes 7 and 8). *In vivo*, in transgenic heat-shocked nematodes, this doubly mutant gene produced an average of 1.4 extra cells in the anterior pharynx, thus weakly but clearly providing protection against programmed cell death (Table 1). This experiment indicates that p35 protects against programmed cell death through a mechanism that requires a CED-3 cleavage site.

Like p35, the cell-death inhibitor cowpox virus protein crmA inhibits ICE protease activity¹⁵ and a variety of apoptotic cell deaths, presumably by blocking the activities of ICE or ICE-like proteases^{16–19}. It has been shown that crmA is cleaved by human ICE, and might act as a competitive ICE inhibitor²⁶. We found that crmA was also cleaved by murine ICE (Fig. 2, lanes 1 and 3) but not by the CED-3 protease (Fig. 2, lane 2). When expressed as a transgene in *C. elegans* under the control of the nematode heat-shock promoters, crmA did not protect against programmed cell death (Table 1). However, by replacing the

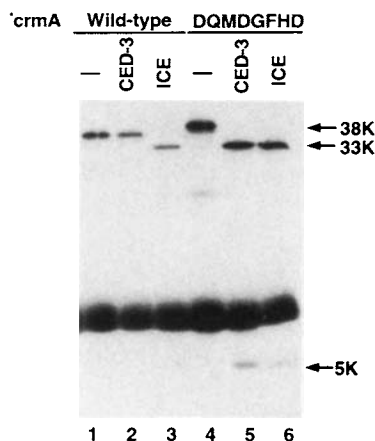


FIG. 2 Cleavage of crmA proteins by the CED-3 and murine ICE proteases. ³⁵S-cysteine-labelled crmA proteins (*crmA) were incubated with 40 ng purified CED-3 or murine ICE protease, both expressed in and purified from *E. coli* (D.X. and H.R.H., unpublished results) at 30 °C for 30 min. Lanes 1–3, wild-type crmA; lanes 4–6, crmA containing the CED-3/ICE cleavage site DQMDGFHD from p35 (see text); lanes 1 and 4, CED-3 buffer; lanes 2 and 5, 40 ng purified CED-3 protease; and lanes 3 and 6, 40 ng purified ICE. The intense band present in all lanes is of unknown origin, and was present when ³⁵S-cysteine was used in the rabbit reticulocyte lysate to label proteins but was not present when ³⁵S-methionine was used (see Figs 1 and 3). We used ³⁵S-cysteine in this experiment to allow visualization of the 5K cleavage product of crmA, which does not contain methionine²⁶. Although full-length mutant crmA protein (lane 4) migrated slightly slower than wild-type crmA (lanes 1 and 2), the cleavage products of this mutant protein were indistinguishable from those of the wild-type protein, for reasons we do not understand.

METHODS. Full-length crmA cDNA was amplified using PCR with plasmid p996 (ref. 10), which contains a full-length crmA cDNA, as a template and primers CAAGCTAGCATGGATATCTTCAGGGAATCG and CAAGAGATCTACTGTGTCATCGTCATCCTGTAGTCATTAGTTGTTGGAGAG. The amplification product was digested with *NheI* and *BglII*, and the resulting fragment cloned into pBluescript KS(+) using its *SpeI* and *BamHI* sites, which have compatible cohesive ends. We used the resulting plasmid pDX10, site-directed mutagenesis and *in vitro* transcription/translation as described in the legend to Fig. 1 to construct crmA mutations and to synthesize ³⁵S-methionine-labelled crmA proteins.

TABLE 1 Requirement of a CED-3 cleavage site for preventing cell death

<i>hsp</i> construct*	Array	No. of animals	Extra cells in anterior pharynx†	Range of extra cells
Vector	1	27	0.2 ± 0.4	0–1
	2	21	0.1 ± 0.3	0–1
p35	1	17	8.9 ± 2.6	5–13
	2	16	8.6 ± 3.5	2–13
	3	21	8.8 ± 3.1	3–16
p35D87E	1	14	0.3 ± 0.5	0–1
	2	19	0.3 ± 0.5	0–1
	3	23	0.3 ± 0.6	0–2
	4	24	0.3 ± 0.6	0–2
p35(1–87) + p35(88–299)	1	26	0.2 ± 0.5	0–2
	2	24	0.1 ± 0.3	0–1
p35D87E K127G	1	22	1.2 ± 0.9	0–3
	2	16	1.6 ± 1.1	0–5
crmA	1	30	0.2 ± 0.4	0–1
	2	22	0.1 ± 0.4	0–1
	3	16	0.3 ± 0.6	0–2
crmA-DQMDGFHD	1	21	4.4 ± 2.6	1–10
	2	33	4.9 ± 2.8	0–10
	3	27	4.9 ± 2.5	1–10

* Plasmids pDX1–p35(1–87) and pDX1–p35(88–299) were made by polymerase chain reaction (PCR) amplification of the p35 coding region from amino acids 1 to 87 and from amino acids 88 to 299, respectively, digested with *NheI* and *KpnI* and cloned into pBluescript KS(+) previously digested with *SpeI* and *KpnI*. Heat-shock promoter (*hsp*) constructs were made by subcloning *XbaI*–*KpnI* fragments from pDX1 and its mutant derivatives and *XbaI*–*KpnI* fragments from pDX10 and its mutant derivative into *hsp* vectors pPD49.83 and pPD49.78 previously digested with *NheI* and *KpnI*. The *hsp* constructs were then co-injected at 100 ng ml^{–1} each into wild-type N2 animals with pRF4 (at 50 ng ml^{–1}), a dominant marker, as previously described²⁵.

† Heat-shock experiments were performed and scored as described previously²⁵. The data shown are mean ± s.e.m. and all depict results obtained after heat-shock treatment.

ICE cleavage site in crmA²⁶ (300-LVADCAST-307) with the CED-3 cleavage site in p35 (DQMDGFHD), we conferred to crmA activity both as a CED-3 substrate (Fig. 2, lane 5) and as a protector against programmed cell death in the nematode. An average of 4–5 extra cells was seen in the anterior pharynx of heat-shocked transgenic animals carrying this mutated crmA gene (Table 1). This finding indicates that protection against cell death in *C. elegans* by the CED-3 cleavage site in p35 does not require the presence of the p35 protein *per se*, and suggests that by changing the sequence around the cleavage site of the crmA protein it should be possible to create specific inhibitors for members of the CED-3/ICE family of cysteine proteases with different substrate specificities.

Because p35 inhibits cell death not only in the nematode but also in insects and vertebrates, we tested whether p35 can be cleaved by four non-nematode cysteine protease activities of the CED-3 family: mouse ICE²⁷, human NEDD-2/ICE^{12,13}, human CPP32/Yama^{11,14} and chicken S/M extract²³, all of which are implicated in causing cell deaths in vertebrates. All four protease activities cleaved p35, and probably all at the same site, because the cleavage products comigrated and because a D87A substitution in p35 either completely prevented or greatly reduced the cleavage reactions (Fig. 3). These observations lead us to suggest that in other organisms the anti-cell-death activity of p35 also requires a cleavage site for CED-3/ICE family proteases.

How might the CED-3/ICE cleavage site in p35 act? It might generate through proteolysis a product or products that can prevent cell death. For example, although our data suggested that coexpression of the 10K and 25K cleavage products of p35

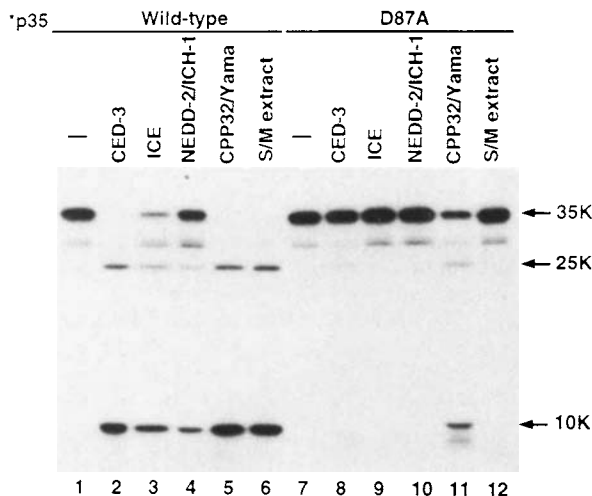


FIG. 3 p35 is a substrate of murine ICE, human NEDD-2/ICH-1, human CPP32/Yama and chicken S/M extract. ^{35}S -methionine-labelled p35 proteins (^{35}S) were incubated with 40 ng purified protease for 20 min at 30 °C. Lanes 1–6, wild-type p35; lanes 7–12, mutant p35D87A; lanes 1 and 7, CED-3 buffer; lanes 2 and 8, purified CED-3 protease (40 ng); lanes 3 and 9, purified murine ICE protease (40 ng); lanes 4 and 10, purified human NEDD-2/ICH-1 protease (40 ng); lanes 5 and 11, purified human CPP32/Yama protease (40 ng); lanes 6 and 12, 1 μl chicken S/M extract²³.

did not generate a functional cell-death inhibitor, it is conceivable that such an inhibitor can be made only by proteolysis of an intact protein. Alternatively, the CED-3/ICE cleavage site in p35 could act by allowing p35 to bind to the active site of, and thereby competitively inhibit, these proteases. If present at relatively high levels, p35 could inhibit by mass action; appropriately high levels of p35 expression might be obtained normally as a consequence of viral infection, and in cell culture and in the worm as a consequence of expression from strong promoters. If cleaved relatively inefficiently, p35 could inhibit by prolonged association with the proteases. We further suggest that crmA, or any other exogenous or endogenous substrates of members of the CED-3/ICE cysteine protease family, might similarly function as competitive inhibitors of these enzymes and hence of programmed cell death. □

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Unbinding force of a single motor molecule of muscle measured using optical tweezers

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THE unbinding and rebinding of motor proteins and their substrate filaments are the main components of sliding movement¹. We have measured the unbinding force between an actin filament and a single motor molecule of muscle, myosin, in the absence of ATP, by pulling the filament with optical tweezers². The unbinding force could be measured repeatedly on the same molecule, and was independent of the number of measurements and the direction of the imposed loads within a range of $\pm 90^\circ$. The average unbinding force was 9.2 ± 4.4 pN, only a few times larger than the sliding force^{3–5} but an order of magnitude smaller than other intermolecular forces^{6,7}. From its kinetics⁸ we suggest that unbinding occurs sequentially at the molecular interface, which is an inherent property of motor molecules.

In the absence of ATP, a myosin molecule binds to an actin filament forming a crossbridge through a rigor bond. On a glass surface coated with a low concentration of heavy meromyosin (HMM), which is a proteolytic fragment of myosin, short actin filaments (1–2 μm long) rotated around a single point over a range of more than 360° (which is analogous to a microtubule tethered by a single kinesin molecule⁹). Long filaments (5–20 μm long) were attached to the surface at nodal points a few micrometres apart (Fig. 1), between which the filament showed brownian bending motion. These observations suggest that each attachment point corresponds to a single HMM molecule¹⁰, although it is possible that a cluster of HMM molecules may have been involved in some cases.

To measure the unbinding force needed to rupture the rigor bond, we used optical tweezers to impose an external load on the crossbridge by manipulating a polystyrene bead attached to the rear end of an actin filament using gelsolin, an actin binding protein (Fig. 1). The actin filament, once detached, could be reattached by manipulating it into the same position on the surface, provided the front remained attached to the surface by one or more HMM molecules. The reattachment was usually made at a slightly different point on the actin filament indicating

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that the unbinding had occurred between the actin filament and the HMM molecule, not between the HMM molecule and the glass surface.

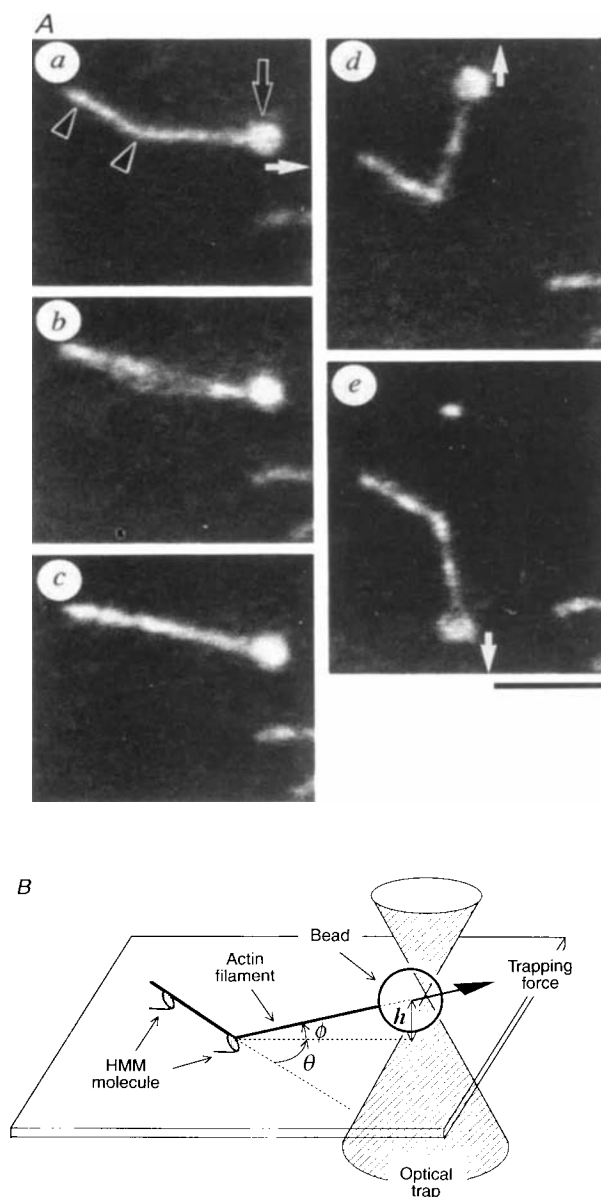
The time course of the displacement of the bead is shown in Fig. 2a. First, the bead smoothly followed a laser spot moving at a constant rate (along the dotted line), but lagged behind after the actin filament became taut (at 1.4 s; compare with Fig. 1A, a), so that a load proportional to the distance between the bead and the trap centre was imposed on the rigor bond (labelled 'stress' in Fig. 2a). When the rigor bond was ruptured, the bead returned to the trap centre (at 2.9 s in Fig. 2a; compare with Fig. 1A, b), and we could directly determine the unbinding force from this abrupt displacement. The distribution of this

unbinding force is shown in Fig. 2c: the peak was at 7 pN and the mean value was 9.2 ± 4.4 pN ($n = 168$). This unbinding force is much smaller than other intermolecular forces, for example 110 pN for actin-actin⁶, 160 pN for avidin-biotin⁷ and >20 pN for actin-gelsolin (judging from the stability observed in the above measurements), but is comparable to the maximum sliding force produced by a single HMM molecule in the presence of ATP³⁻⁵.

Even after the actin filament became taut, the bead was displaced slightly ('strain' in Fig. 2a), such that a stress-strain relation could be obtained (Fig. 2b). The maximum strain at which the rigor bond was ruptured was 69 ± 27 nm ($n = 30$), larger than a myosin head (20 nm), which indicates that the strain contains

FIG. 1 A, A series of fluorescence micrographs showing the measurement of the unbinding force of a rigor bond(s) between a single actin filament and a single HMM molecule. The bead (indicated by the black arrow in a) was trapped by a single beam of laser light and moved at a constant rate to the direction indicated by the white arrow, such that the actin filament became taut. The actin filament was attached to the surface by two separate HMM molecules identified as two nodal points (arrowheads in a)¹⁰. As the laser spot was further moved, the load was increased and subsequently one of the two molecules unbound (b), such that the filament became nearly straight (c). The actin filament could be reattached to the same HMM molecule repeatedly by manipulating the filament into the same position while the second molecule was still attached, and the external loads could be imposed in any direction, as indicated by the white arrows (d and e). Scale bar, 5 μ m. B, Schematic illustration of A: θ is the angle of the applied force, h (<1 μ m) is the height of the bead ($\phi \leq 10^\circ$).

METHODS. Actin and myosin were prepared from rabbit skeletal white muscle. HMM prepared by chymotryptic digestion of myosin was stored in liquid N₂ (ref. 18). Gelsolin was prepared as described previously¹⁹ and crosslinked to the carboxylated polystyrene bead (1 μ m in diameter; Polysciences, PA) with 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (N. Suzuki et al., submitted), such that the barbed end of an actin filament was attached to the bead. The average number of actin filaments attached to the bead was controlled by crosslinking an appropriate amount of bovine serum albumin (BSA) to the bead (for example, BSA:gelsolin = 20:1 in weight ratio). A small amount of BSA labelled with rhodamine X maleimide (Molecular Probes, Eugene, OR) was also crosslinked to the bead surface, and the actin filaments were labelled with rhodamine phalloidin (Molecular Probes). The *in vitro* assay system was based on that reported previously²⁰ but with modifications as follows: the coverslip was exposed to vaporized hexamethyl disilazane (HMDS; Nakalai Tesque, Kyoto) at room temperature overnight. A 50- μ m thick flow cell, of which the lower surface was an HMDS-coated coverslip, was filled with assay buffer (25 mM KCl, 4 mM MgCl₂, 25 mM imidazole-HCl (pH 7.4), 1 mM EGTA, 1 mM dithiothreitol). Then 2–5 μ g ml⁻¹ HMM, in assay buffer, was infused from each side of the cell, at an interval of 60 s, and washed with assay buffer containing 0.5 mg ml⁻¹ BSA, 10 mM dithiothreitol, 0.22 mg ml⁻¹ glucose oxidase, 0.036 mg ml⁻¹ catalase and 4.5 mg ml⁻¹ glucose (solution B). Finally, bead-tailed actin filaments (20 nM actin and 0.05% (w/v) bead), in solution B, were infused. All experiments were done at 28–30 °C after rinsing the flow cell three times with solution B to wash out ATP (≤ 20 nM) carried over from the G-actin solution. An inverted microscope (TMD-300: an oil-immersion objective lens with a phase ring, $\times 100$ NA = 1.3; Nikon, Tokyo) was equipped with optical tweezers based on a 1 W Nd:YLF laser (1053–1000p: $\lambda = 1.053$ μ m; Amoco Laser, IL). The linear polarization of the laser light was changed to circular using a quarter waveplate. The trap centre could be moved at a constant rate by controlling a movable mirror with d.c. servo-motors (optmike-e; Sigma Koki, Hidaka, Japan). A phase-contrast image of the bead was digitized and its centroid was calculated with a frame memory computer (DIPS-C2000; Hamamatsu Photonics, Hamamatsu, Japan). A fluorescent image of an actin filament was simultaneously acquired with a modified dual-view microscopy system^{21,22}. The trap stiffness used was about 0.1 pN nm⁻¹ (at a laser power of 95 mW without the objective) as measured by the following two distinct methods. First, the position of a bead undergoing thermal brownian motion around the trap centre at a low laser power, for example, 0.43 mW, was analysed at a shutter speed of 1/8,000 s with a non-interlace CCD camera (TM-9700; PULNIX, Kyoto). Assuming the Boltzmann distribution for the bead position, the trap stiffness was calculated as 0.44×10^{-3} pN nm⁻¹,



corresponding to 0.097 pN nm⁻¹ at 95 mW. The stiffness values thus estimated at $h = 0.5, 1.5$ and 2.5 μ m coincided to within $\pm 5\%$. Second, the flow cell was displaced at a constant rate using a substage with a piezoelectric transducer (L. Pickelmann, Munich, Germany). A bead, trapped at $h = 2.5$ μ m so as to make the effects of viscous coupling to the glass surface negligible, was displaced owing to the viscosity of water. The displacement was proportional, to 200 nm, to the force calculated as $F = 6\pi\eta av$, where η is the viscosity of water, a is the radius of the bead (0.5 μ m), and v is the velocity of the flow cell. The trap stiffness was 0.095 pN nm⁻¹ at 95 mW.

the elongation or tilting of some elastic parts in addition to a myosin head. It should be noted, however, that the average elastic modulus, $0.58 \pm 0.26 \text{ pN nm}^{-1}$ ($n=17$), estimated at a stress of 8–10 pN (dotted line in Fig. 2*b*), was similar to the elastic modulus of a crossbridge estimated in muscle fibres^{1,11}.

We could repeatedly measure the unbinding force of the same HMM molecule by selecting long actin filaments attached to the glass surface at more than two points (Fig. 1); short filaments attached at a single point were difficult to reattach after unbinding because of extensive brownian motion at the free end. The unbinding force did not change significantly, even with

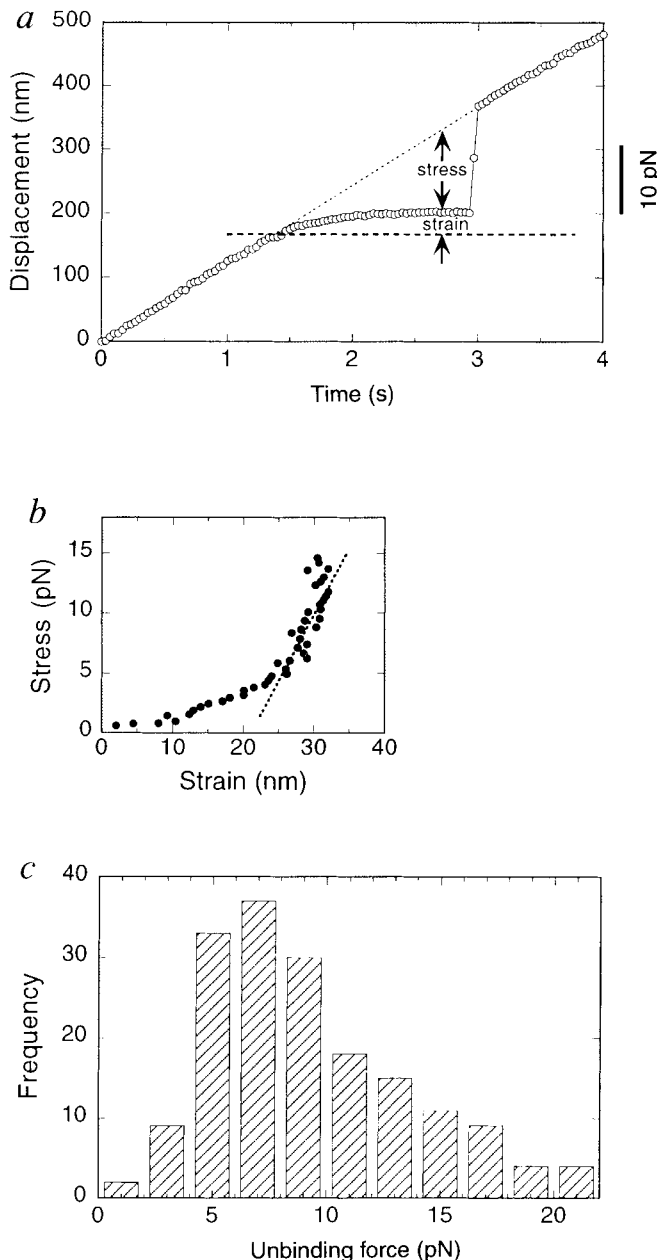


FIG. 2 *a*, Time course of the movement of the trap centre (dotted line, 120 nm s^{-1} , corresponding to about 12 pN s^{-1}) and the bead (circles) to which a single actin filament was attached. From the abrupt displacement at 2.9 s, when the rigor bond was ruptured, the unbinding force between an actin filament and an HMM molecule was estimated. *b*, Stress-strain relation obtained after the actin filament became taut at 1.4 s in *a*. In most samples the elastic modulus increased as the strain became large. The dotted line shows the elastic modulus at the imposed load of 8–10 pN. *c*, Histogram of the unbinding force between a single actin filament and a single HMM molecule.

repetition of the measurements (Fig. 3*a*), indicating that HMM had not been denatured despite the rigor bond being ruptured several times by the external loads.

We also examined whether the unbinding force depends on the direction of the external load (Fig. 1*Aa, d, e*). The angle (θ in Fig. 1*B*) was limited within the range of $\pm 90^\circ$, because filaments forced to bend at angles larger than 90° tended to break at the bend. As shown in Fig. 3*b*, the unbinding force appeared to be independent of the direction of the load. This is due to the flexibility of an HMM molecule, as revealed by the rotation of the attached short actin filaments. In contrast, the sliding force⁴ and velocity^{12,13} of an actin filament depend largely on the orientation of myosin molecules assembled in a thick filament.

The lifetime of a rigor bond without a load has been reported to be 10^2 – 10^3 s (refs 14, 15). In our experiments (Fig. 2), HMM molecules detached within 3 s of an external load being imposed ($1.5 (=2.9-1.4)$ in Fig. 2*a*), implying that the external load decreased the lifetime of the rigor bond. To confirm this load dependence, we applied a sudden, constant load and then measured the time that elapsed before unbinding occurred (Fig. 4). Individual lifetimes measured repeatedly on each HMM clearly show that a higher load tends to shorten the lifetime (see lines connecting the symbols in Fig. 4). This is consistent with the observation that the detachment rate of rigor crossbridges in muscle fibres is stress-sensitive¹⁶. Note that, although variations between different HMM molecules are large, data on individual HMM are relatively consistent (see also Fig. 3).

In our experiments, the actin filament was always 'pulled' in the direction opposite to the 'power stroke' of myosin (the bead being on the rear end of the actin filament), and the reverse pull at $\sim 10 \text{ pN}$ accelerated the rate of unbinding of the rigor bond by a factor of 10^2 – 10^3 (from 10^2 – 10^3 s to ~ 1 s (Figs 2 and 4)). This would imply an interaction distance of the order of $(\ln(10^2-10^3))k_B T/10 \text{ pN} \sim (2-3) \text{ nm}$ (where k_B is the Boltzmann constant and T the absolute temperature)¹⁷, which is an order of magnitude greater than the length of individual bonds at the molecular

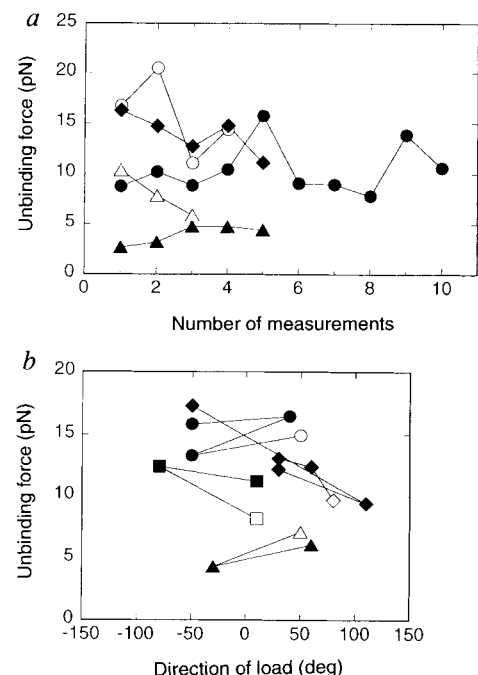


FIG. 3 Effects of the number of measurements (*a*) and the direction (θ defined in Fig. 1*B*) of the external loads (*b*) on the unbinding force measured on the same HMM molecules. Different symbols in each figure represent different HMM molecules. Open and closed symbols in *b* indicate the first and subsequent measurements, respectively, connected by lines.

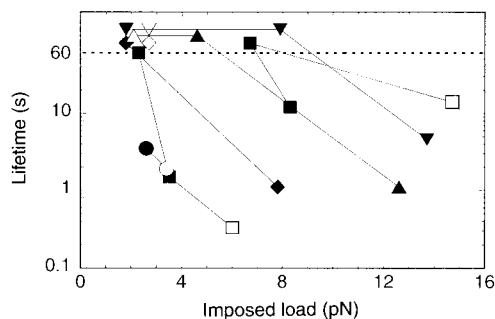


FIG. 4 Effects of the imposed loads on the lifetime of the rigor bond. The flow cell was displaced stepwise within 1/30 s so as to impose a constant external load (2–15 pN) using a piezoelectric substage (p-770.10; Physik Instrumente, Germany) with a function generator (1915; NF Electronic Instruments, Japan). The lifetime was repeatedly measured at various loads for the same HMM molecules. For those rigor bonds not ruptured within 60 s, the lifetimes are plotted above the broken line indicating 60 s. Different symbols represent different HMM molecules, and open and closed symbols indicate the first and subsequent measurements, respectively, connected by lines.

interface. On the other hand, the rate of unbinding of molecular bond(s) having a typical interaction distance (~ 0.3 nm) would be increased only by a factor of $2 \sim \exp(10 \text{ pN} \cdot 0.3 \text{ nm}/k_B T)$ (cf. Fig. 3 in ref. 8). This suggests that the reverse pull of the actin filament decreased very efficiently the activation energy barrier for unbinding.

Taken together, our results indicate that pulling an actin filament tends to distort the actin–myosin interface in such a way as to break the bonds at the interface sequentially from one end. This should be a rapid process compared to unbinding without

a load, in which all bonds must break almost simultaneously. Conversely, if the myosin molecule in the 'power stroke' prestate could be 'pushed' in the direction of the 'power stroke', that is, towards the rigor-like state, a conformational change(s) that progressively increases the cohesive force between actin and myosin would occur and a longer lifetime would be expected. These properties were predicted on the basis of biochemical data, but our mechanical results suggest that they are inherent to the actin–myosin interaction, and that the role of ATP splitting regulate the process of conformational change. □

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A general mechanism for transcriptional synergy by eukaryotic activators

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ONE of the important regulatory concepts to emerge from studies of eukaryotic gene expression is that RNA polymerase II promoters and their upstream activators are composed of functional modules whose synergistic action regulates the transcriptional activity of a nearby gene^{1–3}. Biochemical analysis of synergy by ZEBRA, a non-acidic activator of the Epstein–Barr virus (EBV) lytic cycle⁴, showed that the synergistic transcriptional effect of promoter sites and activation modules correlates with assembly of the TFIID:TFIIA (DA) complex in DNase I footprinting and gel shift assays. The activator-dependent DA complex differs from a basal DA complex by its ability to bind TFIIB stably in an interaction regulated by TATA-binding protein-associated factors (TAFs). TFIIB enhances the degree of synergism by increasing complex stability. Similar findings were made with the acidic activator GAL4-VP16. Our data suggest a unifying mechanism for gene

activation and synergy by acidic and non-acidic activators, and indicate that synergy is manifested at the earliest stage of preinitiation complex assembly.

ZEBRA (also called Zta) is known to stimulate assembly of the DA complex *in vitro*^{1,5}. We reasoned that if this was indeed a biologically relevant event then it should be included in the mechanism of transcriptional synergy⁶. The electrophoretic mobility shift assay in Fig. 1a and the corresponding DNase I footprints in Fig. 1b demonstrate that ZEBRA strongly stimulated formation of DA complexes on two different core promoters bearing multiple upstream sites (3 and 7 sites: Z₃- and Z₇-E4T or -M), but did not alter the basal amount of complex formed on a template bearing a single site (Z₁). The results indicate that the site-dependent synergistic effect of ZEBRA observed in transcription assays *in vivo* and *in vitro*^{1,7} (M.C. and T.C., unpublished observations) is first manifested at the DA complex and that the cooperativity is not restricted to a particular core promoter. Furthermore, synergistic DA recruitment occurs while the ZEBRA sites are saturated, which suggests that multiple molecules of activator are simultaneously interacting with DA, in accordance with a model we have proposed previously^{8,9}. DNase I footprinting also showed that removing either of two non-acidic activation modules (regions I and II), which act synergistically to enhance ZEBRA's activity in transcription assays^{1,7} (data not shown), abolishes formation of the DA complex (Fig. 1c), which is similar to the mutant's transcriptional effects. The ZEBRA-responsive DA footprint encompasses both the TATA box and downstream regions, generating an extended series of downstream enhancements and protections^{10–12}.

ZEBRA promotes binding of TFIIB to DA (Fig. 2). The gel shift in Fig. 2a shows that, although TFIIB had no discernible effect on the mobility or amount of basal DA complex (lanes 1 and 3), it generated a clear DAB supershift in the presence of

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ZEBRA (compare lanes 2 and 4). The effect was specific because the DABZ complex, but not DAZ, could be further supershifted by incubation with affinity-purified TFIIB antibodies (data not shown). TFIIB stimulated complex formation slightly (2.6-fold) when TFIIA and TFIID were made limiting by a short incubation (Fig. 2b, lanes 6 and 7, 15 min), although the effect was weaker with longer incubation times (compare lanes 6 and 7 at 15 and 60 min). TFIIB did not influence TFIID binding in the

absence of TFIIA (lanes 5–8), neither did it elicit a supershift of DA in the absence of ZEBRA (lanes 2 and 3). The stimulatory effect of TFIIB was not an artefact of the gel shift assay because TFIIB also enhanced DA binding in a DNase I footprinting assay (Fig. 2c) when the TFIID concentrations were made limiting. Thus a further increase in the degree of synergy can be obtained by additional factors joining the complex. Unlike the case of DA in Fig. 2a, TFIIB binds to TATA-binding protein

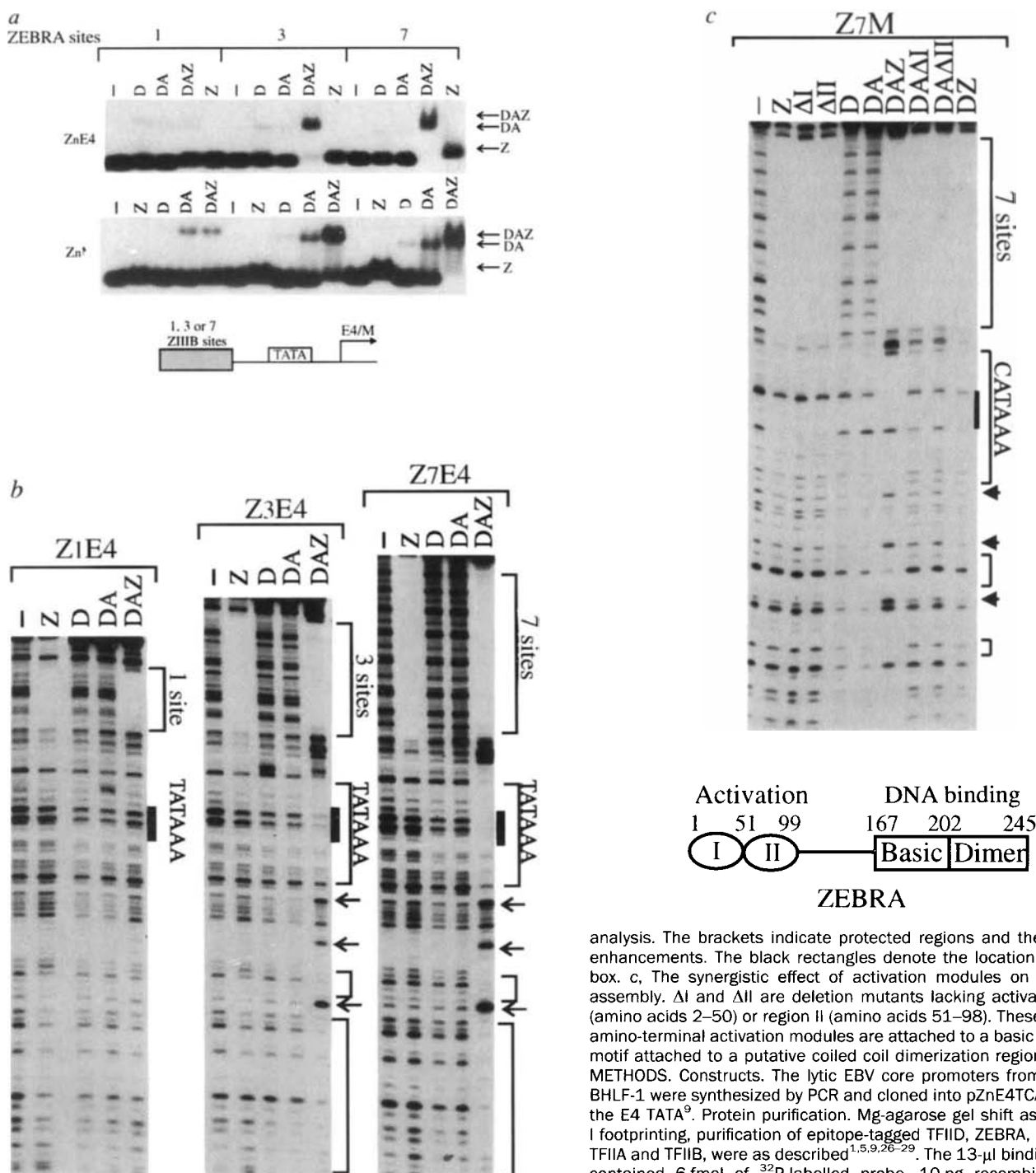


FIG. 1. ZEBRA promotes synergistic DA complex formation. **a**, Gel shift analysis. ³²P-labelled DNA fragments with 1, 3 or 7 high-affinity ZEBRA binding sites (ZIIIB) upstream of the EBV BMRF-1 (Z_{1,3,7}M) or the adenovirus E4 (Z_{1,3,7}E4) core promoters (see the schematic diagram at the bottom) were incubated with recombinant ZEBRA (Z), TFIID (D) and TFIIA (A) and fractionated on 1.4% Mg-agarose gels. **b**, DNase I footprint

analysis. The brackets indicate protected regions and the arrows are enhancements. The black rectangles denote the location of the TATA box. **c**, The synergistic effect of activation modules on DA complex assembly. Δ I and Δ II are deletion mutants lacking activation region I (amino acids 2–50) or region II (amino acids 51–98). These Ala-Pro-Gln amino-terminal activation modules are attached to a basic DNA binding motif attached to a putative coiled coil dimerization region. **METHODS.** Constructs. The lytic EBV core promoters from BMRF-1 or BHLF-1 were synthesized by PCR and cloned into pZnE4TCAT, replacing the E4 TATA⁹. Protein purification. Mg-agarose gel shift assays, DNase I footprinting, purification of epitope-tagged TFIID, ZEBRA, recombinant TFIIA and TFIIB, were as described^{1,5,9,26–29}. The 13- μ l binding reactions contained 6 fmol of ³²P-labelled probe, 10 ng recombinant ZEBRA (~370 fmol), 20 ng of the two recombinant TFIIA subunits (~330 fmol), 160 ng of TFIIB (5 pmol), and 200 ng of holo TFIID (~300 fmol). The TFIIB concentration was roughly fourfold higher than in a previous study⁵. The conditions used in our gel shift and footprinting studies are routinely tested by transcription (see ref. 5). The autoradiographs above were scanned into Adobe Photoshop 3.0 using a ScanMaker III (Microtek) imported into Corel Draw 5.0 and labelled.

(TBP) (T) or a TBP:TFIIA (TA) complex¹³ in the absence of ZEBRA (Fig. 2*d*, compare lanes 2 and 3 with 4 and 5), suggesting that one or more TAFs in TFIID apparently reduce affinity of the DA complex for TFIIB in the absence of activator. ZEBRA moderately enhances (<2-fold) T or TA complex formation in the presence of TFIIB (compare lanes 2–5 with 6–9), suggesting that TFIIB may be a direct target of ZEBRA consistent with affinity chromatography data (data not shown).

Site-dependent enhanced binding of DA is a general phenomenon of both non-acidic and acidic activators (Fig. 3*a, b*). In DNase I footprints (Fig. 3*a*) and gel shift assays (Fig. 3*b*) the acidic activator GAL4-VP16 (G) stimulated DA complex formation poorly on a single site but had a strong effect on a five-site template, and multiple activators were required for recruitment of TFIIB (Fig. 3*b*). Note, however, that the stabilizing effect of TFIIB on the GAL4-VP16 complexes, although apparent, is weaker than that observed with ZEBRA (data not shown).

A model summarizing our results, and incorporating recent studies that have identified candidate targets of ZEBRA and GAL4-VP16^{5,14, 17}, is given in Fig. 4. The ability of TBP to sup-

port relatively high levels of transcription suggested the possibility that TAFs might act as inhibitors in the absence of activators⁶. However, our experiments demonstrate that TAFs play diverse roles (reviewed in ref. 18) in synergy: a positive role leading to activator-stimulated DA complex formation, and a negative role reducing the affinity of TFIIB for TFIID in the absence of multiple activators and TFIIA. In this regard the TAFs increase the net stimulation by the activator, much like the upstream stimulatory activity coactivator fraction¹⁹.

A previous study by Choy and Green showed that TFIIB could associate in crude HeLa extracts with promoters bearing only a single bound GAL4-VP16 molecule²⁰, with synergistic

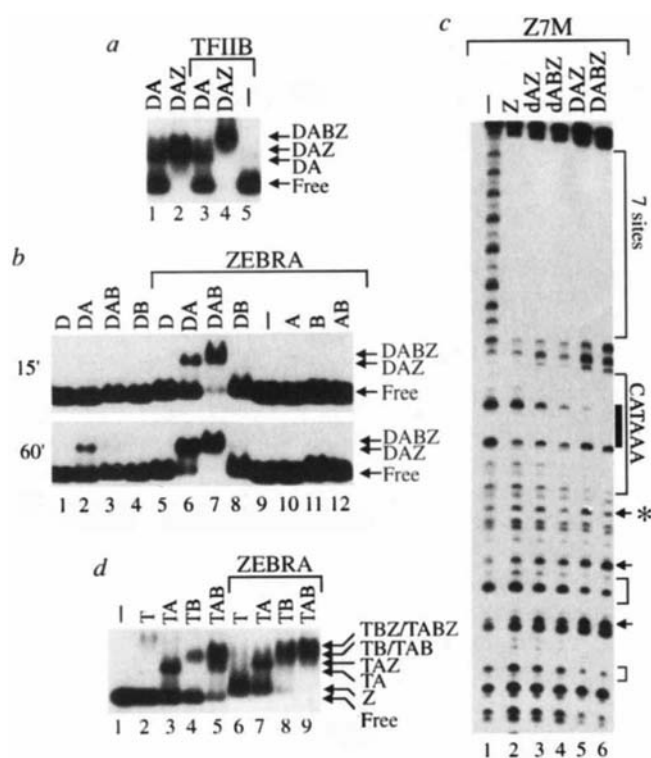


FIG. 2 Binding of TFIIB to the DA complex requires activator and is regulated by TAFs. *a*, TFIIB supershifts a DAZ complex formed on multiple sites. The binding mixtures were as described in Fig. 1 but with the addition of TFIIB where indicated. The probe was Z7H. The positions of the DAZ and DABZ complexes are distinguished by arrows. *b*, TFIIB stimulates complex formation when DAZ is limiting. The binding mixtures contained the indicated components and ³²P-labelled Z7M but were incubated for 15 min and the standard 60 min to determine the effects of time on the DAZ complex. Similar results were obtained by limiting the concentration of DA instead of time (data not shown). *c*, DNase I footprint showing the effects of TFIIB on ZEBRA-stimulated DA complex formation at low (d) and high (D) amounts of TFIID on Z7M. The asterisk indicates an increased protection in the presence of TFIIB. *d*, TFIIB binding to TBP. The binding mixtures contained the indicated components and were performed as in Fig. 1. T refers to complexes formed with 10 ng of recombinant human TBP. TFIIA (2.0 ng) was used to form TA complexes. The arrows indicate the mobility and composition of the various complexes.

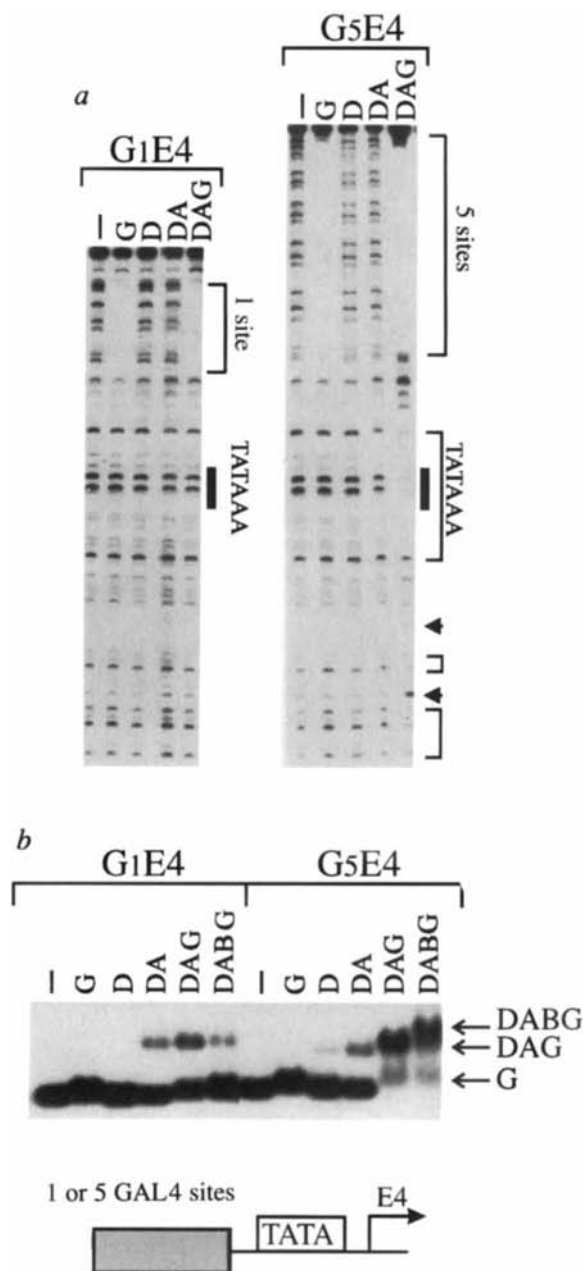
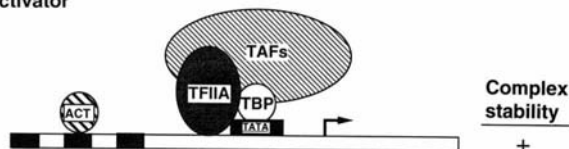


FIG. 3 The acidic activator GAL4-VP16 stimulates DA complex formation. *a*, DNase I footprints of GAL4-VP16-stimulated DA complexes. The binding reactions were performed as described in the Fig. 1 legend and contained the indicated components and 10 ng of recombinant GAL4-VP16. The templates contained 1 or 5 GAL4 sites upstream of the adenovirus E4T core promoter⁸. *b*, Gel shift analysis of GAL4-VP16-stimulated DA and DAB complexes. The positions of the GAL4-VP16 (G) complexes with DA (DAG) and DAB (DABG) are indicated with arrows.

Basal DA complex: 0 or 1 activator



Synergistic DA complex: multiple activators

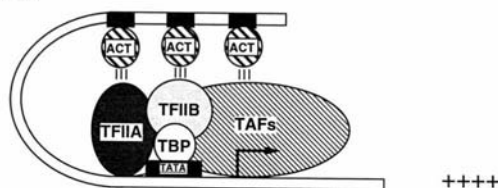


FIG. 4 A general model for gene activation and synergy. Both acidic and non-acidic activators stimulate DA complex formation by interacting with multiple members of the complex. This productive interaction is signalled by a TAF-dependent conformational change leading to an extended DA footprint and recruitment of TFIIB. A single molecule of activator is unable to promote either efficient DA complex formation or recruitment of TFIIB owing to the negative action of TAFs.

activation occurring at a step subsequent to TFIIB recruitment²⁰. In contrast, our study suggests that multiple activators, either ZEBRA or GAL4-VP16, assemble the DA complex and subsequently recruit TFIIB in a synergistic fashion. It is plausible that the conditions used in the previous study caused DA binding in the absence of activator, inadvertently bypassing the recruitment we routinely observe. Moreover, the binding of TFIIB only to multiple-site templates in our study may reflect a more stable, and hence probably more functionally relevant, interaction with DA because the Mg-agarose gel is more stringent. This idea would not contradict Choy and Green²⁰, who found that the TFIIB bound to a single-site template generated an inactive complex. Consistent with this idea, TFIIB failed to enhance DA recruitment on a template bearing only one site.

Our DNA binding experiments are largely supported by earlier kinetic data on ZEBRA and GAL4 action using transcription and open complex assays^{1,5,21}. Furthermore, our results are in broad agreement with *in vivo* studies showing that TFIIB binding is rate limiting and that an activator affects the kinetics of this process^{22,23}. We suggest that the effects observed here, superimposed upon the cooperative binding of activators and the general factors to chromatin^{24,25}, could explain the large synergistic transcriptional responses observed *in vivo*. □

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CORRECTIONS

An efficient prebiotic synthesis of cytosine and uracil

Michael P. Robertson & Stanley L. Miller

Nature **375**, 772–774 (1995)

IN this report on the prebiotic synthesis of cytosine from cyanoacetaldehyde and urea, related work that should have been cited was omitted. The prebiotic synthesis of cytosine from 1 M cyanoacetylene and 1 M urea in 4.8% yield was reported earlier by J. P. Ferris, R. A. Sanchez and L. E. Orgel (*J. molec. Biol.* **33**, 693–704; 1968). Replacing cyanoacetylene by its hydrolysis product cyanoacetaldehyde gives essentially the same yield, suggesting that the cyanoacetylene reaction may have gone through cyanoacetaldehyde. The concentrated urea solution (20 M) postulated in a drying-lagoon model of prebiotic synthesis results in yields of cytosine as high as 53%. □

Short alanine-based peptides may form 3_{10} -helices and not α -helices in aqueous solution

Siobhan M. Milick, Gary V. Martinez, Wayne R. Flori, A. Paul Todd & Glenn L. Millhauser

Nature **359**, 653–655 (1992)

THE originally reported ESR spectrum for the 3K-(4,8) peptide exhibited artificially sharp features due to the presence of a monoradical contaminant. The peptide has since been repurified and the proper spectrum reported¹. Linewidth measurements indicate that the biradical interaction between the spin labels is weaker in the repurified 3K-(4,8) peptide than in the 3K-(4,7), which continues to support the original observation that $d(4,7) < d(4,8)$. Thus, the conclusion of the original paper—that the 3K peptide contains a significant proportion of 3_{10} -helix—remains intact. We are indebted to M. D. Rabenstein and Y. K. Shin for bringing this matter to our attention and for suggesting improved methods of peptide purification.

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ERRATUM

Peripheral deletion of antigen-reactive T cells in oral tolerance

Youhai Chen, Jun-ichi Inobe, Reinhard Marks, Patricia Gonnella, Vijay K. Kuchroo & Howard L. Weiner

Nature **376**, 177–180 (1995)

PANELS a–c of Figs 2 and 3 of this Letter were accidentally transposed during the production process. □

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Commentary articles deal with issues in, or arising from, research that are also of interest to readers outside research.

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Abbreviations, symbols, units and Greek letters should be identified the first time they are used. Acronyms should be avoided whenever possible and, if used, defined. Footnotes are not used in the text.

Acknowledgements are brief and appear after the reference list; grant and contribution numbers are not allowed.

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Immunitie, impunitie

Frances M. Brodsky

Immunity. Editor Benjamin Lewin. Cell Press. 12/yr. USA \$385, elsewhere \$455 (institutional); USA \$95, elsewhere \$165 (personal).

THE several definitions of the word 'immunity' all refer to a state of privileged exemption from rules and regulations, from natural and physical dangers or from social obligations and constraints. These definitions seem applicable to the new journal *Immunity*. In his inaugural editorial article, Benjamin Lewin explains that the title *Immunity* means that coverage will extend "beyond the formal definition of immunology, and into all systems that contribute to, or interact with, the immune response of the organism". Another stated goal is to explore the cutting edges of immunology, but not necessarily to be comprehensive. Work published in *Immunity* is thus to enjoy privileged exemption from traditional constraints.

During its first 18 months, *Immunity* has been successful in accomplishing these goals and has been well received by immunologists. A privileged offspring of Cell Press, the journal was born with a silver spoon in its mouth and some of its success is attributable to good breeding. The papers have the same top-quality sexy appearance of those in *Cell*, and a 3–6-month turnaround from submission to publication. The mini-reviews, like those in *Cell*, are topical, concise and written primarily by leaders in the field. *Immunity* has been launched with strong submissions from its stellar editorial board, setting a standard for high-quality publications.

The papers in *Immunity* contribute significantly to immunology, but are marginally too specialized for the popular general journals. The well-established *Journal of Experimental Medicine* has long provided this forum for immunologists, and in a recent revitalization has broadened its scope and bias from traditional cellular and disease-related immunology to include molecular immunology. The contents of *Immunity* look like a selective raid on the contents of *Journal of Experimental Medicine*, picking out the more trendy papers with molecular orientation. *Immunity* also covers some systemic immunology, a field beginning to benefit from knockout and transgenic systems. Interestingly, the raid by *Immunity* has not depleted the number of top-quality papers in *Journal of Experimental Medicine*, whose contents (about triple that of *Immunity*, which plans not to exceed 15 papers per issue) are supplemented by solid, informative papers that are still a cut above the good, but pedestrian fare in the *Journal of Immunology*. The availability

of fodder for *Immunity* suggests it has filled a niche.

In their respective introduction and conclusion to the proceedings of the 1989 Cold Spring Harbor symposium on immunology, both Charles Janeway and Jonathan Howard perceived immunology as entering a new era, following the resolution of several of its great mysteries.

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

Protecting veil: coloured scanning electron micrograph of a human lymphocyte. The microvilli projecting from the cell surface play a part in cell motility.

That new era is reflected in the content of

Immunity. With some understanding of the molecular and genetic basis for immunity, the void between phenomenological and 'paradigm'-setting papers can now be filled. Some might say that the excitement has gone, whereas others would say that the field has matured to a state of real experimentation instead of relying on description, theory and serendipity. *Immunity* is publishing papers from those areas of experimentation that are being most hotly explored. Although an immunologist should still consult *Journal of Experimental Medicine* and *Journal of Immunology* for breadth and depth, *Immunity* represents a finger on the pulse of the field.

There are two other definitions of 'immunity' that raise a cautionary note regarding this journal and others of its type. 'Ecclesiastical immunity' refers to sanctuary from invasion by secular armies. Possibly because of its title, *Immunity* may evade readers outside the field, such as cell and developmental biologists who would find some of its papers relevant. Finally, an obscure use of the word 'immunity' (attributed by the *Oxford English Dictionary* to Latimer's sermon before Edward VI in 1549) warns of potential consequences of extreme pressure to publish. "There is sum place in London, as they saye *immunitie*, *impunitie*. What should I call it? A preueleged place for whoredom." Fortunately, this definition does not apply to *Immunity* or, hopefully, to the other new journals reviewed here. □

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New Journals 1995

CRITERIA for journals to be considered for review in this issue were circulated to publishers earlier this year, and were also published in *Nature*. They were that:

- (1) the first number appeared during or after June 1993 and at least four separate numbers were issued by the end of May 1995 (although some of the journals eligible for, but not covered in, last year's review issue were also considered)*;
- (2) the journal is published at least three times a year;
- (3) the main language used is English;
- (4) where possible at least four issues should be made available for review, including the first and the most recent numbers.

The time criteria ensure that a reasonable sample of issues is available for judgement by the time reviews are commissioned.

Several journals known to satisfy the criteria were not submitted for review, or arrived too late for inclusion. It proved difficult to find reviewers for other, doubtless worthy journals, while some titles were considered to be of marginal interest to *Nature's* audience. Journals covering any aspect of science were

eligible, although those dealing with clinical medicine and pure mathematics were excluded, as were abstracts publications. A list of eligible titles submitted for review but not covered appears on page 272.

The brief given to the reviewers was to limit themselves to comments on the publications sent to them, and to avoid discussion of general questions of periodical publishing. Opinions expressed in the reviews are based on a sample of issues, usually the first, the most recent (as of the end of May) and two in between. As in previous years the preponderance of journals in the biological sciences reflects the bias of the material submitted.

Details of editors and frequency of publication, and the subscription rates appearing at the top of each review, are given in most instances for 1995. This information is not complete in all cases, and readers interested in subscribing to a particular journal should check the rate with the publisher concerned. □

*See *Nature* 371, 440–458 (1994); 365, 569–589 (1993); 359, 435–464 (1992); 353, 457–481 (1991); 347, 581–599 (1990).

Grant engineering

Richard Vile

Gene Therapy. Editors Karol Sikora, Joseph Glorioso, Bob Williamson and Theodore Fridmann. *Stockton*. 6/yr. Europe £145, elsewhere £155 (institutional); \$65 (personal).

Cancer Gene Therapy. Editors Robert E. Sobel and Kevin J. Scanlon. *Appleton and Lange*. 4/yr. USA \$190, elsewhere \$215 (institutional); USA \$85, elsewhere \$108 (personal).

THE prospect of therapy is justification enough for researching the molecular genetics of just about any disease. As well as providing a warm glow of altruistic satisfaction, a very real advantage is that such research allows grants to be written with a distinctly therapeutic bent. So the advent of gene therapy into the clinic, albeit currently for drastic diseases and desperate patients, has led to an explosion in the number of research papers, reviews and clinical protocols — all of which must find a home in hard copy. These two journals are responses to this boom.

The journals share a similar structure, combining typically up to 6 or 7 original research papers with a general review, a literature survey, editorials and updates on clinical protocols that have been applied for or accepted. In addition, both journals cover annual meetings with publication of abstracts, providing a useful way to keep up to date with research ahead of the lag between submission of manuscripts and their appearance in print. For the most obvious difference one need look no further than the titles. *Gene Therapy* accepts

manuscripts covering advances relevant to any disease for which gene therapy holds out promise. So although cancer features strongly, other genetic diseases are also covered in both primary research and review articles. By contrast, *Cancer Gene Therapy* restricts its output more to papers directly on cancer its problems. Nevertheless, the reviews often deal with topics that have wide applicability in gene therapy as a whole (such as viral vectors for gene delivery) and there would be as strong a case for, say, cystic fibrosis therapists to subscribe as for molecular oncologists.

The broader appeal of these journals to sceptics of gene therapy, or researchers with less applied interests, is more uncertain. Both journals are concerned with papers directed essentially at therapists rather than on those reporting advances in our understanding of the molecular genetics of disease. On the other hand, gene-therapy die-hards would argue that the therapeutic end of the molecular genetics ultimately provides its own justification. In addition, the problems associated with the delivery of genes to cure disease cover a wide range of disciplines, including virology, genetics and immunology. That both journals have now survived their first year, despite having to compete with the highly respected *Human Gene Therapy*, testifies to their acceptance by the gene-therapy community itself. It would seem prudent for most molecular geneticists to follow this trio of journals, if only to provide a little therapeutic boost to their grant applications. □

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Redox redux

Joe M. McCord

Redox Report. Editors John W. Eaton, Nicholas H. Hunt and Simon P. Wolff. *Churchill Livingstone*. 4/yr. USA \$310, Europe £200, elsewhere £202 (institutional); USA \$155, Europe £100, elsewhere £102 (personal).

REDOX Report focuses on free-radical research and oxidative processes as they apply to "biology, medicine, and all aspects of the human environment". At first glance one may question the need for another speciality journal in the relatively small (but still growing) arena of free-radical biology. Its competition is supplied by *Free Radical Biology and Medicine* and *Free Radical Research* and perhaps by *Archives of Biochemistry and Biophysics*, which is less specialized but increasingly somewhere to look for research in the field. Of the 1,380 papers published in 1994 on the subjects of superoxide or superoxide dismutase, I was surprised to find that only about 6 per cent of the papers appeared in these three journals. There seems to be more than enough activity to support a new publication.

What does *Redox Report* have to offer? The editorial policy stated in the inaugural issue is both thoughtful and provocative, and is recommended reading for all editors of peer-reviewed journals. Effort will be made, we are told, to choose reviewers who are early- to mid-career scientists — those who still have the time and inclination to be at the bench. Effort will also be made to ensure that papers on a particular subject or by particular authors do not always go to the same referee. This is a problem that has concerned me as an editor, reviewer and author, but it seems to be a matter for which few journals have a formal policy. It is crucial in a rapidly evolving specialized area that the biases and prejudices of a handful of pioneers should not be allowed to dominate the area, regardless of how valuable their own contributions may have been.

The first four issues offer papers covering the same interesting array of topics that one might expect from *Free Radical Biology and Medicine*. In addition to the preponderance of research articles, each issue so far has included some sort of paired point-counterpoint articles, illustrating the editors' determination to provide a forum for examining issues from all sides. Hypothesis articles are encouraged, as one of the journal's proclaimed goals is to spark the genesis of new ideas in the field. Even though biomedical journals tend to be serious business, an editorial such as the one entitled "Sleepy radicals" proves that even editors can have a sense of humour.

The layout is attractive and accessible.

Furthermore, the journal is taking the bold step of supporting electronic submission and the review of manuscripts by electronic mail. E-mail is a concept whose time has come, promising to reduce the time and costs associated with the editorial process. There will be glitches of course, but ultimately we will all be grateful to those who force us to make use of this efficient new technology.

Despite my concern about the continuing proliferation of new journals, I am confident that *Redox Report* will succeed. I hope it retains its refreshing personality in the process. □

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Science and art of biomolecules

Colin Blake

Acta Crystallographica Section D: Biological Crystallography. Editor-in-chief J. P. Glusker. Munksgaard. 6/yr. Dkr1,985 (institutional); Dkr550 (personal).

Nature Structural Biology. Editor Guy Ridgihough. Nature Publishing, New York. 12/yr. \$495, £300 (institutional); \$195, £125 (personal).

Chemistry and Biology. Editors Stuart L. Schreiber and K. C. Nicolaou. Current Biology. 12/yr. \$480 (institutional); \$120 (personal); \$60 (student).

OF the twin poles of biomolecular science, what we now call molecular biology and structural biology, the latter is a particularly multidisciplinary field bedevilled by a widespread literature. These three journals represent a reasonably orthodox selection of the important part of this literature. Two are the offshoots of long-established journals that have expanded recently into specialized areas to cover the burgeoning field of structural biology, and one is entirely new, devoted to "crossing the boundaries" between medicinal chemistry and the biomolecular sciences.

Acta Crystallographica was established nearly 50 years ago to focus international discussion on the problems of crystallography. At the time, crystallography dealt almost entirely with 'small' molecules, but even the first volume had a short paper on myoglobin. So the appearance now of *Section D* of *Acta*, subtitled *Biological Crystallography*, seems rather late in the day. Virtually unchanged in format from that adopted by *Acta* since its inception, *Section D* has the austere look of the essentially technical journal that it is. Having decided to seek its readership through the quality of its contents rather

than glossy display, its one concession to modern times is a sprinkling of discreet colour plates. *Section D* deals mainly with full research papers and contains a few short communications and an occasional editorial article or review. The papers are mostly devoted to the technical development of biological crystallography as a branch of applied physics and to the results thereby obtained. The quality of this literature is high and the expectation is that any significant advance in X-ray techniques will appear first in *Section D*. To that extent, the journal accurately plots the technical advance of what is still the most powerful tool in structural biology. A particular highlight of *Section D* is the proceedings of key conferences, published as a complete series of full-sized refereed research papers. Those on direct methods and crystallization of biological macromolecules are essential reading for those in the field. In keeping with the image of *Acta*, *Section D* is expensive, and publication is slow (6 months on average), but it is a uniquely valuable publication.

Despite the merits of *Section D*, one has to turn elsewhere for the latest results and the biological context of structural analyses. This 'elsewhere' could be any of half-a-dozen journals, but one of the first places to look would be *Nature Structural Biology*. Splitting off two years ago from *Nature* to provide space for the increasing number of papers in structural biology, it has created a lively and handsome home for some of the most important recent papers in structural biology. It has a *Nature*-like format of 'Editorial', 'News and Views', 'Reviews' and 'Correspondence' sections, followed by 7-8 full original contributions. The lavish use of colour is a particular advantage in getting the structural information across. The journal is, however, in competition with other established periodicals, even possibly from within its own stable. Its News and Views articles on the striking horseshoe ribonuclease inhibitor and the DNA gripper transcription factor refer to their original publication in *Nature*: either *Nature* has greater drawing power for the very best papers or it exercises preferential rights over its junior. Nevertheless, with a time of 2-3 months from submission to acceptance, an offer of free colour illustrations, a modest cost and the *Nature* prestige, *Nature Structural Biology* attracts many good research papers, making it one of the few really essential journals in structural biology.

Finally *Chemistry and Biology*. First published a year ago, it has the stated aim of promoting a chemical approach to understanding the biomolecules revealed by structural and molecular biology. The approach is by way of medicinal chemistry, so toxicity, disease and structure-based drug design loom large among the topics covered. In design, the journal is close to its stable companion *Structure*, a competi-

tor of *Nature Structural Biology*. It begins with a review ('Crosstalk'), intended to provoke, and this is followed by reviews and mini-reviews and 4-6 full research papers. Colour is fairly widespread but appears mostly in cartoons and modelled structures: the thrust of the journal is on the use and understanding of biological structures rather than on their experimental determination. This emphasis can make the science seem a little soft at times, particularly in comparison with *Acta*, and sometimes the artwork seems to take over the science. On the other hand, the understanding and exploitation of biomolecules can be rightly claimed to constitute the most challenging area of biomolecular science, and *Chemistry and Biology* may provide an important focus for capitalizing on the enormous investment being made in structural and molecular biology. Offering almost instantaneous publication at a relatively low cost, *Chemistry and Biology* deserves to do well. □

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Fusion science?

John Armstrong

Molecular Membrane Biology. Managing editor Jack A. Lucy. Taylor and Francis. 4/yr. £97, \$160 (institutional); £49, \$80 (personal).

THE operative question today for any new journal is not "Should your library take it?" but "Which journal should you drop in its favour?" In this respect *Molecular Membrane Biology* is off to a good start, because it is a reincarnation of *Membrane*

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Membrane system: electron micrograph of rough endoplasmic reticulum.

Biochemistry. The change of name reveals much of the editors' laudable intention: to cover membranes "from the biophysics of membrane components to the cell biology of their functions".

This is a bold attempt at tackling a vertical section through biology. The increasing progress 'downwards', as cell biology falls prey to molecular methods, means that the subject is becoming more physical. A good example is the process of fusion of vesicles with their target membrane in eukaryotes: most of the participating proteins are probably known, but understanding how they cause lipid bilayers to fuse is a distinctly biophysical problem. So there is a clear niche for a journal that can speak to biophysicists, biochemists and cell biologists in the same language.

Is *Molecular Membrane Biology* such a voice? Perhaps not yet, but there are encouraging signs. The balance of papers so far, understandably considering the journal's origins, seems skewed away from the cell-biological end (four random issues contain three light- or electron- micrographs of cells), but there have been good contributions on membrane protein structure and assembly. The general format of papers is conventional apart from having

experimental procedures at the end, an irritating feature copied from some trendy journals. (Whose idea was it that a result is best appreciated if the reader has no idea how it was obtained?) Time from acceptance to publication is 4–6 months, creditable considering both the apparent absence of electronic submission and the quarterly publication rate (it is the editors' avowed intention to increase this frequency).

One questionable policy was the decision to devote an issue to a meeting: not only did the issue appear some ten months after the event but the publishable contents of the meeting would presumably have appeared anyway. Incredibly, the first page is a verbatim welcome from the conference manager, beginning "Good morning, I'm Phil. . ." and ending "I will not take any more of your time. . .".

Such lapses will doubtless not recur. *Molecular Membrane Biology*, unlike many new journals, is a good idea, and with vision and ambition it should earn a precious place in the library. □

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those 'ologies' whose names are evidently too venerable to utter: h*st*l*gy and c*t*l*gy. So, although not stated explicitly, the chief criterion for publication in *Cell Vision* appears to be that vision may not be practised on cells before they are dead. Those of us who choose to apply our vision to living cells should not feel smug, however, because the range and depth of information now obtainable from dead cells using the sophisticated techniques reported in *Cell Vision* are truly impressive. □

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Letters and paper

Mike Gidley

Carbohydrate Letters. Editor-in-chief Pierre G. Sinay. *Harwood Academic*. 6/yr. ECU54, \$65.

Cellulose. Editor-in-chief John C. Roberts. *Chapman and Hall*. 4/yr. USA and Canada \$230, Europe £135, elsewhere £145.

Dead cells give up their secrets

Graham Dunn

Cell Vision: Journal of Analytical Morphology. Editor-in-chief Jiang Gu. *Eaton*, 154 E. Central St, Natwick, Massachusetts 01760, USA. 6/yr. USA \$125, Europe \$145, elsewhere \$150.

HUMPTY-Dumpty might well have said of the name *Cell Vision* that "it means just what I choose it to mean, — neither more nor less". The new journal's subtitle — *Journal of Analytical Morphology* — would seem to be more informative were it not that any paper submitted from the Great Beyond by the founding father of analytical morphology, D'Arcy Wentworth Thompson, would very probably be rejected by the editors. In fact, potential contributors to *Cell Vision* should know that its editor-in-chief does not use morphology in its defined sense, to mean the study of biological shape or form, but apparently takes it to signify the location of various molecular species in tissue sections and cell smears.

The clearest way of understanding which disciplines the journal serves is by scanning its contents. The editor-in-chief has himself reverted to this foolproof technique in the first issue of Volume 2, where he states that comprehensive review articles have covered the topics of *in situ* polymerase chain reaction (PCR), immunogold-silver staining, confocal microscopy, microwave technology and

antigen retrieval, while research and technical topics have included self-sustained sequence-replication-based *in situ* DNA amplification, reverse-transcription *in situ* PCR, computer-assisted cytopathological diagnosis and modified immunohistochemical procedures.

Overall, the review articles (4–8 pages) and technical articles (3–10 pages) are highly informative and lavishly illustrated (at only \$200 per colour plate) and the first issue seems to be a good laboratory manual for those wishing to experiment with *in situ* PCR, an exquisitely sensitive technique for detecting and locating foreign genes in tissues and cells.

Research articles (3–12 pages) have a strong bias towards clinical pathology, as does the editorial board, and case reports (1–6 pages) provide a forum for "morphologic observations on human or experimental samples, preferably using modern morphologic methodologies". The five editors have obviously put a lot of hard work into launching the journal, contributing no fewer than 16 articles, 9 proceedings and 15 abstracts of their own to the 6 issues that I scanned, but it is to be hoped that their Herculean effort need not be sustained as circulation increases.

During my survey it became clearer that "modern morphologic methodologies" refers to the latest cell- and tissue-labelling techniques descended from

CARBOHYDRATES are structurally complex and diverse molecules that can have a profound and pervasive influence on life processes. They are increasingly attracting the attention of researchers in both biological and physico-chemical sciences, as confirmed by the appearance of these two new journals.

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Roll-ups: paper manuscripts for recycling.

Carbohydrate Letters seeks to provide a home for new developments across the panoply of disciplines and covers all classes of carbohydrates. Papers are invited in camera-ready form to lessen delays between acceptance and publication. In practice, although some papers appear within a month of acceptance, most are published after a delay of six months or so. So far, the primary subject areas covered lie at the chemistry-biochemistry interface, with particular emphasis on synthesis strategies. The quality of articles is high and compares favourably with established journals in the field. A publication

of this type may well find a niche as the natural home for short reports of new insights and methods that have wide relevance but which would sit uneasily as subsections of more substantial papers published elsewhere.

It is perhaps appropriate that there is now a journal devoted to the main component of paper, at least until the relentless march of electronic information rids us of the joys of holding journals in our hands. *Cellulose* sets itself a narrow scope in that it deals with reports only on cellulose and its derivatives; lignin and hemicellulose, the two materials with which cellulose is most commonly associated in nature, are specifically excluded. Production and presentation are excellent, and the quality of articles is high. There is a mix of detailed review articles and full research papers, although only 18 contributions in total were published in the first year.

Cellulose has enormous potential as a renewable raw material, and one could argue that a dedicated journal is needed to serve the community of scientists devoted to cellulose and its derivatives, as well as the many industries (pulp, paper, fabric) dependent on these materials. Whether *Cellulose* becomes such a house journal will depend on whether experts in the field would rather instead submit good-quality manuscripts to journals with a broader remit. For so specialized a field, *Cellulose* could provide a perfect vehicle for comments on articles in other journals concerned with cellulose, but evidence of such community-building is so far sadly lacking. This apart, *Cellulose* is off to a promising start. □

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No empty vessel

Peter Clark and Tony Firth

Endothelium: Journal of Endothelial Cell Research. Editor-in-chief G. M. Rubanyi. Harwood Academic. 4/yr. ECU462, \$601 (companies); ECU296, \$385 (libraries); ECU99, \$129 (personal).

ALTHOUGH some might argue that a journal devoted entirely to one cell type is too narrow in scope, others may feel, given the interesting biology and wide-ranging clinical issues concerning endothelial cells, that it is surprising that a journal called *Endothelium* has only recently appeared. *Endothelium* publishes invited reviews, mini-reviews, full research papers, short communications, conference reports, technical notes and correspondence. It aims to cover all aspects of endothelial biology, including the effects of endothelium-derived

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Flesh and blood.

jargon-ridden. The research papers are generally concise and of good quality, although the really big stories in endothelial cell research are still more likely to be found in broader and more influential journals.

The journal adopts the standard double-column layout, and the text and diagrams are readable and clear. There is, however, still room for improvement in the quality of the reproduction in micrographs. There are no page charges, and colour illustrations can also be included for free. The publishers even go one step further by making 'negative page charges': principal authors receive credit (ECU15 or \$20) for each article, which individuals or institutions can put towards the purchase of any of the publisher's products. Here surely is a practice that should be encouraged!

The increase in endothelial research in recent years has created a niche for a new journal. *Endothelium* might fill it, at least in part. Other vascular journals, such as *Microvascular Research* and *Circulation Research*, overlap in areas of content, but *Endothelium* could provide a forum that brings together information of a variety of types, and as such would provide a focus for endothelial research. Much will depend on the ability of the journal to attract a reasonable share of high-quality papers in basic endothelial cell biology. We urge workers to consider sending their work to *Endothelium* and hope that the journal flourishes. □

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agents on other systems. The members of the editorial board, including several distinguished names, represent this broad scope.

So far, a wide range of topics has been featured, although there is an apparent bias towards endothelial products and their effects on target cells. As expected in a new journal, the quality of the papers is variable. Some of the reviews are excellent and of broad interest, whereas others are over-specialized and

Recipes at a glance

Andrew Griffiths

Molecular Biotechnology. Editor-in-chief John M. Walker. Humana. 6/yr. USA \$180, elsewhere \$200.

THIS journal is difficult to pigeonhole. Published as Part B of *Applied Biochemistry and Biotechnology*, it aims to publish protocols for nucleic-acid and protein manipulation. These are sensibly presented in a step-by-step cookbook style, removing the need for users to decode tortuous prose into working instructions. They also contain useful warnings about potential pitfalls and troubleshooting suggestions. This is the sort of priceless time-saving information that one can get from a colleague down the corridor but which is rarely found in the sanitized methods that appear in most other journals.

The protocols themselves are something of a mixed bag, and arguably some are rather too trivial to merit publication. Most issues also contain one or two review articles. I particularly enjoyed the review on "Critical Assessment" of catalytic antibodies by Tawfik and colleagues in the first issue, which discusses the limitations of antibodies as enzymes, something that many authors in this field tend to gloss over.

The few original papers are definitely the journal's weakest aspect and it is not clear if the editors intend them to constitute an important section or appear as something of an afterthought.

Molecular Biotechnology is not a journal to which I would take out a personal subscription, although I would certainly scan it on a visit to the library. □

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TEN out of ten

Simon Wolff

Toxicology and Ecotoxicology News: International Reviews, Opinions and Updates in Toxicology, Environmental Toxicology and Ecotoxicology. Editors Sheila O'Hare and Chris Atterwill. Taylor and Francis. 4/yr. £115, \$193 (institutional); £45, \$76 (personal).

Now here is a nice little journal. Taylor and Francis have decided to publish a popular reviews and opinion journal on toxicology, in all its aspects. *Toxicology and Ecotoxicology News (TEN)* contains no original papers but is very well constructed

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

Slick operation: clean-up operation of the rocky coastline of Knight Island, Alaska, after the Exxon Valdez oil-spill in 1989.

and will have broad appeal.

At the time of writing, my radio tells me that the French have finally tested their nuclear bomb in the South Pacific. Fuss was made about the immorality of pulling such a stunt so far from their own backyard. But justification for the fuss was based on the supposed effects on human health. Similarly, when a well-known oil company recently tried to dump some unwanted hardware in the Atlantic, a lot of noise was made about heavy metals and organic carcinogens accumulating in the food chain. Open any newspaper, any day, and there is a story about real, possible or imagined injury through environmental exposures. Worrying about being poisoned by some villain seems fundamental to human nature.

Unfortunately, it is only recently that toxicologists have become more aggressive in their mission to explain their subject, long seen as the poor relative of pathology and pharmacology. *TEN* represents the maturing of that determination. The journal contains leading articles, subject reviews, legislative matters, general news in the field, book reviews and news of meetings. The reviews are the real meat and provide good background reading in a variety of areas. Areas covered (taken at random) include bacterial endotoxins (accounting for two per cent of premature mortality worldwide); modelling of biological toxicity through structure-activity relationships (part of attempts to reduce animal testing); immune reactions of agrochemicals (of interest to food faddists and those with a serious interest in multiple chemical sensitivity); and the metabolism of chemicals (such as cosmetics and mineral oils) by skin. The level of these review articles is such that they are of benefit to active researchers while providing good background material for, say, undergradu-

ate projects. The material covered in the reviews, opinion pieces and open discussion sections, intelligently explores the wide range of toxicology. Legitimate areas of coverage range from the endogenous toxins associated with burn injury and kidney failure to the regulation of water contamination by chemicals toxic to fish.

Is the journal a good buy? It is certainly well put together and worth, in my opinion, the asking price for four issues. It will be even better value when publication goes bimonthly at the end of this year. The journal should be prescribed reading for everybody involved in the broad area of toxicology and adverse reactions. Because the review articles are useful to undergraduate and postgraduate students in a variety of disciplines (from biochemistry to zoology), the journal should be on the shelf of most university libraries. In many ways *TEN* steals the thunder from Elsevier with its range of *Trends* journals. Elsevier managed *Trends in Biochemical Sciences* (*TIBS*) and *Trends in Neurosciences* (*TINS*) but, for reasons which totally escape me, failed to produce *Trends in Toxicological Sciences*. It relegated the area instead to a subsection of its pharmacology journal. Taylor and Francis have filled the gap. □

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Journal prices

Details of editors and frequency of publication, and the subscription dates given at the top of each review are in most instances for 1995. Readers interested are therefore advised to check prices with the publisher before subscribing.

SPL Environmental briefing

Andrew Jordan

Human Environment: The International Environmental Policy Analysis Newsletter. Edited by Ingar Palmund. *Gron Ide HB, Stockholm*. 12/yr. USA \$85, elsewhere \$110.

It can be frustratingly difficult keeping abreast of events in such a fast-moving and wide-ranging field as the environment. Policies constantly change as politicians come and go; new and potentially costly legislation is adopted and implemented; and, behind all of this, scientific understanding continues to accumulate, often gradually but sometimes abruptly. *Human Environment* joins several publications that have sprung up in recent years to provide a ready briefing service for researchers, business people and bureaucrats who may not have the time, or resources, to consult the specialist literature or follow policy-related events as closely as they might wish.

Human Environment is published monthly. Each issue contains around eight pages and is fronted by a one-page leading article that normally deals with a topical event or set of issues. The bulk of each edition consists of short, abbreviated reviews of recent magazine or newspaper articles drawn from mainstream publications (for example *Science*, *Nature*, *New Scientist* and *The Economist*) and the broadsheet press. Each issue also normally carries a one- or two-page 'focus' piece that explores a particular policy issue in greater depth. Early issues have also included a discussion of resource management problems in the Baltic Sea, a couple of book reviews, an interview with a United Nations official and a series of 'viewpoints' from experts.

Human Environment is attractively produced and considerably cheaper than some of its potential rivals. But unlike *Global Environmental Change Report*, *International Environmental Reporter*, *Environment Watch*, *Environmental Data Services Report* or *Energy, Economics and Climate Change*, it simply does not provide the detailed coverage and in-depth analysis of events and personalities that more discerning readers may be looking for. It seems unlikely that *Human Environment* will flourish if it continues to reproduce articles that can be easily located and electronically retrieved at the press of a button, or are mainstream enough to be picked up by anyone who takes a daily broadsheet newspaper. □

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The emperor's new rag

Jeffrey Gray

Journal of Consciousness Studies. Executive editors J. A. Goguen and Robert K. C. Forman. Imprint Academic, PO Box 1, Thorverton, Exeter EX5 5YX, UK. 4/yr. \$48, £28 (institutional); \$25, £15 (personal).

NEW scientific journals commonly meet a need spawned by novel methods or by the opening up of a fresh field to experimental investigation. *Journal of Consciousness Studies* is a clear exception. There is as yet no agreed method for studying consciousness, nor even any consensus that it is yet (or ever?) amenable to scientific investigation. This new journal has emerged, rather, from a change in the *Zeitgeist*: consciousness is no longer taboo. As an example of this change, in 1971 I published a paper on consciousness that elicited just two requests for reprints; a quarter of a century later I am a welcome guest at conferences where I say exactly the same things! Has the field moved on, even if I have not? Is it yet part of the 'art of the soluble' (in Peter Medawar's lapidary phrase)?

The editors neatly duck this question by titling their journal "Controversies in Science and the Humanities" and setting its scope to cover "all aspects" of consciousness, including psychology, neuroscience, physics, philosophy, artificial intelligence, and social, cultural, ethical and religious issues. The result is a heady brew, although neither psychology nor neuroscience so far figures much in the mixture. This is a pity, for it is here that speculation is likely eventually to find its strongest empirical constraints. In fact, experimental data of any kind hardly figure in the first four issues. In the one exception, an intriguing report (by Nunn *et al.*) of the effects on psychological performance of being hooked up to an electroencephalogram machine, the methods and results of the experiments are discreetly tucked away in an appendix. What there is most of so far is, perhaps surprisingly, physics, with Roger Penrose's quantum-gravity theory holding centre stage (inspiring *inter alia* the paper by Nunn *et al.*); and, less surprisingly, philosophy, mostly alas going over very familiar ground.

The contributions about mystic experiences and theological issues avoid the wilder shores and, for the most part, provide education (or sometimes entertainment) even for the hard-headed (so long as they are not so hard-headed as still to suppose that the problem of consciousness is not a problem at all). So, do we need this journal, even if no-one is yet sure how to make the problem of consciousness soluble? Yes, we do: there is no other journal quite like it, and one

day we shall, I think, look back to its appearance as a defining moment when the prologue to the real play (whatever that may turn out to be) began. And, at the price, it's a snip! □

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The Santa Fe telegraph

Karl Sigmund

Artificial Life. Edited by Christopher G. Langton. MIT Press. 4/yr. USA \$135, Canada \$161.57, elsewhere \$151 (institutional); USA \$45, Canada \$65.27, elsewhere \$61 (personal); USA \$25, Canada \$43.87, elsewhere \$41 (student/retired).

SANTA Fe is to artificial life what Vienna has been to psychoanalysis. Through a sequence of well-timed moves (international workshops, a polished series of proceedings, popular books), Christopher Langton has established a tight-knit community of a-lifers that now seems strong enough to warrant a journal of its own. This is of course how any new discipline grows: what seems specific to both a-life and ψ -a, however, is the pivotal role played by one founding father, the strong impact on the *Zeitgeist* and, coming from

less well-meaning colleagues, persistent doubts about whether or not these fields belong to science.

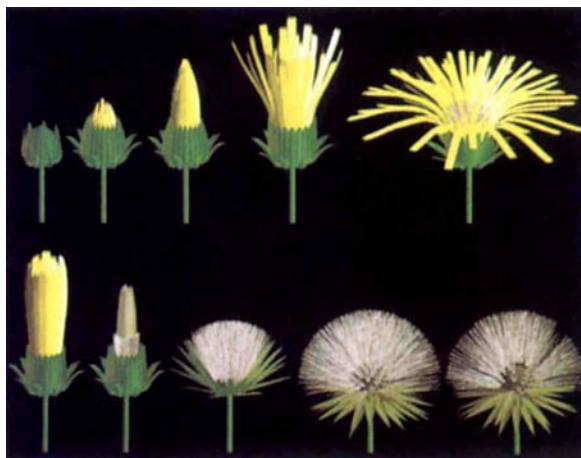
The editor-in-chief of *Artificial Life* is Langton himself (no other choice would be conceivable). The editorial board is essentially a fair cross-section of the usual visitors to the Santa Fe workshops — almost all regular contributors to the proceedings volumes (*Artificial Life: Vols I-III*) which did so much for the field. Langton, who is considerably more easy-going than Freud ever was, allows a remarkable profusion of topics to be included in his journal. The first three numbers, written by members of the editorial board, serve to stake out the claims in a vast domain that covers hardware, software and wetware alike, dealing with computer viruses, genetic algorithms, bioengineering, community construction, artificial intelligence, adaptive behaviour, molecular evolution, self-assembling toolkits, pattern formation, robotics and so on, the whole lot liberally sprinkled with philosophical comments on ethical and social questions.

Taken together, these overview articles would make a solid successor to the three proceeding volumes, and leave no doubt that this highly talented set of authors could keep turning out more of the same. But that, of course, is not the point: as Langton writes, the introductory articles are merely meant to prime the pump. It is too early yet to know if a steady flow is running. The first 'regular' contributions are encouraging: their overall tendency, however, is to stake out still more claims rather than to dig deeper. In the long run, that can be dangerous. Sooner or later the field will have to be circumscribed. It is not just the sum of what the crowd of insiders is doing.

Although Langton refrains from defining artificial life (speculating that "perhaps in ten years or so" it might be possible), he writes that the bread and butter of the journal will consist of papers on computational approaches to

open problems in biological theory and in the application of biological principles to engineering. So far there seems to be too little of the former. If this is a sign of continuing sympatric speciation, it would rob the new field of an important opportunity: to study evolution through thought-experiments in a context free of historical contingencies.

For a-life's aficionados, the journal is a must. Theoretical biologists, empirical mathematicians and the readership of *Physica D* will also do well to follow *Artificial Life*.



Flower power: development of the hawkweed flower *Hieracium umbellatum*; from *Artificial Intelligence: An Overview* ed. C. G. Langton (MIT Press, \$42, £24.95).

The price is reasonable. The journal offers scope for letters, articles, reviews and reports. Graphics and colour plates are, not unexpectedly, of high-gloss quality. To some it may seem odd that a journal on a discipline so cheerfully close to science fiction is still published as hard copy: but it is paired with an on-line electronic bulletin board called "ALIFE". Let us hope that the implicit challenge to computer hackers and virus breeders will remain resistible. □

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Inner visions

James V. Haxby

Human Brain Mapping: A Journal Devoted to Functional Neuroanatomy and Neuroimaging. Editor-in-chief Peter T. Fox. Wiley-Liss. 4/yr. USA \$190, Canada and Mexico \$230, elsewhere \$245 (institutional); USA \$80, elsewhere \$90 (personal).

INTEREST in research into the functional organization of the human brain has been heightened over the past decade by the use of high-resolution, three-dimensional brain-imaging methods for measuring local haemodynamic changes reflecting patterns of neural activity. These methods can be used to map higher cognitive functions of the intact

satellite of the International Society for Cerebral Blood Flow and Metabolism Meeting, which attracted more than 800 participants.

Human Brain Mapping, which was launched in 1993, has the potential to provide an integrated forum for this rapidly developing interdisciplinary field. Although the most innovative and groundbreaking work will probably continue to appear in leading general science and neuroscience journals, *Human Brain Mapping* promises to provide a home for reports now scattered among dozens of journals in psychology, neurology, psychiatry and physiology.

Unlike many new journals, *Human Brain Mapping* will make it easier for investigators to find reports of relevant research by workers in different disciplines. The need for such a journal is apparent, and already another journal, *NeuroImage*, has altered its editorial policy to become a second, integrated forum for brain-mapping research, and promises to augment interdisciplinary communication further by including reports on brain mapping in nonhuman species.

Until recently, the method of choice for this work has been positron emission tomography (PET). Because of the cost and complexity of this method, research has been limited to a handful of well-funded and productive centres. The recent development of magnetic resonance imaging (MRI) methods for measuring haemodynamic changes promises to open up the field. Functional MRI allows neuroscientists to engage in human brain-mapping research using scanners already installed for clinical imaging, and so require a fraction of both the cost and the staff necessary for PET studies. Although functional MRI is still dauntingly difficult to master, it will enable many more centres to engage in functional brain-imaging research. The first fruits of this methodological advance are just starting to appear in the literature.

Further expansion in human brain-mapping research comes from the rehabilitation of other methods of noninvasive study of human brain function, such as electro- and magnetoencephalography. These methods have been plagued by problems in identifying the location of structures that generate signals, but they clearly offer temporal resolution on a timescale more appropriate for mapping sequential events in the human brain. It is hoped that these methods can be successfully integrated.

The early issues of *Human Brain Mapping* have included reports using all these methods, as well as reports of mapping research on postmortem human brains and of new methods of analysis. The large format and high-quality reproduction allow the dramatic colour images — a hallmark of the field — to be shown to magnificent effect. Stunning cover illus-

trations also make this an eye-catching journal for the office and coffee-table. □

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Neurobiological networks

Jack Cowan

Journal of Computational Neuroscience. Editors John Rinzel, James M. Bower, Eve Marder, Idan Segev, Charles Wilson and John P. Miller. Kluwer. 4/yr. Dfl474, \$270 (institutional); Dfl165, \$75 (personal).

WORK on neural models and networks started effectively in the 1930s. At that time, the only specialized journal partly

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Neural integration: nerve cell on silicon chip.

devoted to these topics was *Bulletin of Mathematical Biology*, founded by N. Rashevsky of the University of Chicago. It was in this journal that McCulloch and Pitts published their famous 1943 paper making the connection between neural networks and logic. After the Second World War, there was a clearly recognized need for other outlets, triggered in part by developments in cybernetics applied to brain research. The result was another journal, *Kybernetik*, which appeared in 1961 and was later renamed by Springer as *Biological Cybernetics* to appeal more to Anglo-Saxon librarians. Its chief editor was W. Reichardt. For several years this was the principal specialized journal for theoretical and computational neuroscience.

But in the early 1980s, developments in the theory of neural networks, particularly John Hopfield's work on symmetric weight networks, and especially the so-called 'backpropagation' network built by David Rumelhart, Geoffrey Hinton and Ron Williams, led to an avalanche of new work, and computational neuroscience and artificial neural networks came into focus as a distinct field. Not surprisingly, several different specializations have

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Mind matter: MRI of an adult human brain.

human brain, such as language and memory, as well as sensory and motor functions. Because of the development of new functional brain-imaging methods accessible to more and more neuroscientists, and the rehabilitation of other methods for measuring brain function to compensate for some of the inherent shortcomings of these imaging techniques, we are on the brink of an explosive growth in human brain-mapping research. Workers in this endeavour come from diverse areas in medicine, psychology and the information sciences. The health of the new field was evident at the first annual Brain Map Meeting, held last June in Paris as a

since developed, each with a more-or-less separate journal.

One finds, for example, that physicists influenced by Hopfield's work tend to publish in journals such as *Network*, *Physica D* and *Physics Review E*. Computer scientists, engineers and mathematicians, perhaps more heavily influenced by 'backpropagation', tend to publish either in *Neural Networks*, the official journal of the International Neural Network Society, or else in *Neural Computation*, which also accepts papers in theoretical neuroscience and whose chief editor is Terrence Sejnowski. This journal has strong links with the Neural Information Processing Systems Foundation, which sponsors an annual meeting in Denver, Colorado, the (refereed) proceedings of which form an archival record of progress in the field.

What about neurobiologists? Several years ago, under the leadership of James Bower, a new meeting on computational neurobiology was organized, devoted mainly to the properties of single neurons and small networks, ion channels and so on. This soon became a successful annual event, full of talks in which results obtained using well-known simulation software such as GENESIS and NEURON are presented. The forum for publication of these findings is *Journal of Computational Neuroscience*, first published last year. The journal already has a character distinct from those of the other journals mentioned above. Its contents very definitely concern neurobiology, not cybernetics or automata theory, or artificial neural networks, or algorithms and architectures, or implementations. On the basis of my reading of the journal so far (I am in fact a subscriber), I think it is already the best place in which to publish extensive computational and theoretical studies of neurobiological networks. It overlaps a little, but does not compete, with *Neural Computation*, in my opinion the leading periodical dealing with all the other topics as well as with some neuroscience.

The journal itself is well produced, as are all Kluwer periodicals. The format is excellent, the figures and tables are nicely done and the refereeing seems sufficiently rigorous. The subscription price is reasonable. What more can one ask? I expect *Journal of Computational Neuroscience* to become firmly established over the next decade as one of the main outlets for work in computational neuroscience. □

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Journal prices

Bibliographical details at the top of each review are given in most instances for 1995. Potential subscribers are therefore advised to check prices with the publisher.

Calculating the secrets of life

Rakefet Rosenfeld

Journal of Computational Biology: A Journal of Computational Molecular Cell Biology. Editor-in-chief David T. Kingsbury. Liebert. 4/yr. USA \$174, elsewhere \$218.

The Journal of Computational Biology — for which a more suitable name might be *Journal of Biological Computations* — is aimed at filling a void created by the migra-

nable that the complexity and amount of biological data generated in the past two decades are challenging even the more theoretically minded computer scientists. The journal's founding editors, David T. Kingsbury (editor-in-chief) and Michael S. Waterman (senior editor), two of the principal driving forces behind the migration, have therefore taken on an extremely difficult mission: the creation of a truly interdisciplinary journal.

Truly interdisciplinary works that precisely state the biological problem in both biological and computer-science terms, rigorously work out the mathematics involved and go on to apply the algorithms developed in order to answer the biological

question are rare. In the first volume of the journal (containing four issues) the interdisciplinary goal is successfully met in several papers, commonly those written by an interdisciplinary team. A clear example is a paper entitled "On near-optimal alignments of biological sequences" by D. Naor (a computer scientist) and D. L. Brutlag (a biologist) that will undoubtedly interest computer scientists and biologists alike. Many other reports in these first volumes also aim to achieve this goal, but more often than not it is clear that the biology is alien to the authors and the result is a computationally sound but biologically less interesting paper. There are also many papers in which the connection to biology seems almost coincidental and is briefly stated in a single sentence; biologists, even the most computationally oriented, will find little interest in these. As the proportion of the former type of papers increases at the expense of the latter, the journal could well become the preferred 'home' for truly interdisciplinary studies in the field of molecular biology. □

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Model makers: James Watson and Francis Crick display the structure of DNA.

tion of computer scientists into the biological sciences. The void is situated between the more mathematical journals such as *Bulletin of Mathematical Biology* and *Mathematical Bioscience* and the more biological journals such as *CABIOS*. In the former category, rigorous mathematical analyses of computational methods with potential applications to biological questions are presented with brief discussions of the biological problems and usually no mention of direct applications or biological results, whereas in the latter category, reports on computational methods and applications are presented with an amateurish treatment of the mathematics, at best using heuristic or empirical arguments.

Journal of Computational Biology aims to address — and sometimes answer — biological questions with computational tools that have been rigorously developed and analysed. In the words of the opening editorial article, "biological science is now posing questions that not only require computational solutions, but which also provide problems of fundamental interest to computer scientists, statisticians, and mathematicians, creating an environment for effective interdisciplinary work". Although some would argue that the migration of these workers into biology is due to more prosaic reasons such as funding, it is unde-

Barrier breaker

Stephen B. Dunnett

Learning and Memory. Managing editor Judy Cuddihy. Cold Spring Harbor Laboratory Press. 6/yr. USA \$127, elsewhere \$137 (institutional); USA \$57, elsewhere \$67 (personal).

In their introduction to the first issue, the editors of *Learning and Memory* adopt the ambitious mission of spanning topics "ranging from human amnesia to gene expression, from systems neurobiological studies of learning to synaptic plasticity in disassociated culture". They therefore aim to encompass a broad range of levels of

analysis from the most basic molecular and cellular aspects of neurobiology to applied research and human clinical studies. The unifying factor in this multidisciplinary approach is the common theme of understanding the principles of learning, crossing the conventional barriers that have separated cellular and molecular neurobiology on the one hand from cognitive and behavioural science on the other.

This is a challenging mission, and I admit that I opened the first issues with scepticism. The format is fairly standard for a journal of this type: 4–6 research reports per issue, with a smattering of reviews and so far one technical note. Presumably, if a successful flow of manuscripts is established, the thickness of the individual issues will expand. The quality of printing and reproduction is modern, crisp and clear.

So far, so good, but not enough to justify yet another new journal. The impressive feature of the first issues, however, is the editors' success in attracting an outstanding selection of manuscripts across the full range of disciplines encompassed by the journal. Not only are these initial reports of a consistently high experimental quality, but the authors have been successfully encouraged to write with a clarity and style that should make the journal readily accessible to its intended multidisciplinary readership.

The label 'technical note' is deceptive, as the article in question turns out to be a full-length methods paper that provides the detailed rationale, technical description and results of using a computer-controlled, removable on-demand escape platform as an innovative modification of the Morris water maze. The modification offers a powerful way to resolve learning and motivational factors in the performance of animals in the task. Such technical developments often pave the way to important theoretical advances, and I hope that the editors will continue to solicit further technical notes of this standard.

The success of the journal in attracting a broad and sophisticated range of articles is no doubt a reflection of the large board of editors of international calibre and repute. Not surprisingly, many of the articles in the early issues come from members of the board.

If the editors can elicit a flow of similar excellent work from the wider readership, maintaining the breadth of coverage and a stringent review programme to sustain the quality, then *Learning and Memory* could become the journal of choice for articles in the field. □

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Engineering the oceans

Brian McCartney

Journal of Marine Environmental Engineering. Editor-in-chief Michael S. Bruno. Gordon and Breach. 4/yr. ECU83, \$90.

MARINE scientists are well aware of the myriad applications and roles of the ocean, ranging from a resource of food, energy and unique organisms to a source of coastal floods, a medium for bulk transport, a reshaper of coastlines, a depository of waste, a sink for pollution and a mediator of climate. Reaping of the ocean's benefits, protection from its dangers and avoidance of adverse long-term effects of

analysis of stresses in the ship's structure that mid-ocean cycle of deballasting-reballasting is not a safe option, but that continuous flushing is. It is possible that journals of neither marine microbiology nor marine architecture would be comfortable with these cross-disciplinary papers, and so *Journal of Marine Environmental Engineering* fulfils a real need.

Five papers address aspects of numerical modelling applied to coastal transport processes, to oil spills in Arctic waters, to the plume of a sludge discharge and to the use of a shell approach, including GIS (geographical information system) and visualization tools to aid environmental management, especially of coastal zones. These papers all concentrate more on applications than on the kind of mathematics and theory generally found in journals of fluid dynamics and numerical methods.

Papers on engineered devices include accounts of a water blade to remove surface oil and a wave-driven artificial upwelling system. A review of more than 20 years of US research into ocean thermal-energy conversion concludes that, using off-the-shelf technology, an operating plant could be built as an environmentally sound alternative to fossil fuel. As a by-product, the deep ocean water brought up for the heat cycle could be used for other purposes such as aquaculture, air conditioning and refrigeration.

Optical and acoustic sensing of suspended and ponded sediments are described in several papers with relevance to water quality and disposal practices. Future possible ocean monitoring of planetary waves on basin-wide scales using acoustic tomography is also considered in one paper, and the costs and benefits of detection of climate change are addressed in another. These papers were invited by the editors to stimulate discussion. Such short communications to this debating forum are published at the first opportunity after editorial review. All other papers are peer reviewed and the quality of the content is high, although there is some evidence of inadequate proof reading. The journal's format is A5, with good-quality colour plates bound in at the back of each issue; this is not as inconvenient as it seems, because the figures are also reproduced as half-tones next to the text of the papers themselves. Model results in particular benefit from the use of colour.

Journal of Marine Environmental Engineering serves a valuable purpose in providing academic engineers with a publishing outlet for marine papers. Because these cover such a wide spectrum of applications, however, it is difficult to say whether the journal will find a dedicated readership in the various marine industries.

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Water-resistant: Thames flood barrier in London.

its exploitation require knowledge of marine science, and especially marine environmental engineering, a discipline that often differs greatly from land environmental engineering. *Journal of Marine Environmental Engineering* aims to provide a forum for this interdisciplinary field. It unashamedly caters for the applied papers "that describe the use of science and engineering as a *tool* in the solution of present and future marine environmental problems".

The interdisciplinary intent is exemplified by two papers in the first volume that deal with the problem of global transport of unwanted microbiological organisms through the ballast water of ships. Chemical and physical methods of killing toxic dinoflagellate cysts are considered, the most effective way turning out to be heat treatment: a heat exchanger, possibly from the engine-cooling circuit, could be used to treat the ballast water as it is loaded. The other paper studies the microplankton in a ship's ballast water, and concludes from an

Beaming the laser into the home

Christopher Whitehead

Journal of Laser Applications. Editor-in-chief Sidney S. Charschan. *Chapman and Hall*. 4/yr. USA and Canada \$159, Europe £110, elsewhere £125 (institutional).

This year is the thirty-fifth birthday of the laser. Far from being a scientific curiosity in its infancy, the device is now approaching the staid respectability of middle age and the associated responsibility of having to work for its living. Lasers are now widely used in many areas of science, engineering, medicine and manufacturing and are even commonplace in the home; anyone who has a CD player or a CD-ROM drive owns a laser. A journal devoted to applications of lasers might seem to be overdue. *Journal of Laser Applications* is the official journal of the Laser Institute of America (and is free to members) and was started in October 1988 as "the major forum for cross-fertilisation of ideas in disciplines applying laser technologies". In 1994, Chapman and Hall began to publish a revamped version of the journal to "expand its circulation into a wider international market". Four issues are now published a year.

Each issue has an introductory section with a leading article, reports and information of direct interest to members of the Laser Institute. The rest contains invited overview articles, book reviews and submitted papers that are subject to peer review. There are about 10 articles in each issue with publication times of 3–6 months. Articles are accompanied by figures (often poorly reproduced) and photographs (in monochrome). Wisely, the editorial policy of the journal is to concentrate on certain definite areas of laser applications. These declared areas are materials processing, medicine, surgery and bioeffects, sensing, measurement and control, and laser safety. Inevitably, most contributions come from the United States but there is a significant number of articles from workers in Japan and Europe. Articles dealing with materials processing are in the majority. The Laser Institute is keen to promote safe practice in the use of lasers and the journal serves a useful purpose in bringing articles on laser safety to the attention of the user community in a way not possible for similar articles published in journals devoted to safety and occupational health. There is also an interest in the use of lasers in education and most issues have papers under the theme of "Applying Lasers in the Classrooms". These form a particularly useful collection of articles for teachers and lecturers concerned with developing practical classes involving lasers.

The journal's style is reminiscent of *Applied Optics*, published by the Optical Society of America, although *Applied*

Optics has a much wider coverage than just laser applications. *Journal of Laser Applications* generally contains two or three articles of interest in each issue, so it is well worth looking at in the library. It certainly fills a gap by publishing papers on laser safety, lasers in education and general review articles, but I doubt

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Light work: surgeon with laser torch.

whether leading international researchers using lasers in materials processing or medicine, say, will ever submit their first-rank contributions to the journal. The support of the Laser Institute will probably ensure its survival, and institutions with significant numbers of laser users might well contemplate taking out a subscription. □

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Surface active

David King

Surface Review and Letters. Editor-in-chief S. Y. Tong. *World Scientific*. 6/yr. \$390 (institutional); \$190 (institutions in developing countries); \$120 (personal).

Interface Science. Editor-in-chief David J. Srolovitz. *Kluwer*. 4/yr. \$216, Dfl414 (institutional); \$95, £225 (personal).

Tribology Letters. Editors-in-chief Nicholas D. Spencer and Wilfred T. Tysoe. *Baltzer*. 4/yr. Sfr354.

It is hardly a surprise that these three new specialist journals dealing with solid surfaces should be launched now. They all lie within the general area covered by the

journal *Surface Science*, launched by Elsevier in 1963 and considered at the time to be a highly specialized journal in a fledgling field of science bordering on physics, chemistry, materials science and engineering. It proved to be a resounding success, recently given a triumphal celebration by the publishers with the appearance last year of a special volume, "Surface Science: The First Thirty Years". This period marked the development of the field from a qualitative, macroscopically based subject to one based on a detailed knowledge of surface atomic geometries, dynamics and electronic charge distribution. These developments have come hand-in-hand with major transformations in commercially important technologies, particularly in the manufacture of microelectronics but also in the manufacture of new materials generally, including the design of catalysts. The worldwide impact has been enormous and it is my view that the journal itself has played an important role by providing a focus for this activity.

In its first full year of publication, 1964, *Surface Science* ran to 574 pages. By last year this had risen to 8,600 pages, and Elsevier had brought out two companion journals, *Applied Surface Science* and *Surface Science Reports*. The journal *Langmuir*, published by the American Chemical Society, covering the broader field of surfaces and colloids, had also been successfully launched.

Surface Review and Letters must be seen as a direct competitor to *Surface Science*. As Elsevier is, alas, a notoriously expensive publisher, and World Scientific seems to be capable of undercutting it, this is a welcome move. The coverage in the first year of operation, running to 700 pages, meets the editor's stated objective to include both the physical and the chemical properties of surfaces and interfaces. But because both the editor and his associate editor, Dilano Saldin, hail from a physics department and the board of editors is very heavily biased towards physics, it will be interesting to see whether the essentially interdisciplinary character of the field can be fully represented. There is also among members of the board an undue emphasis on structural determination; although this will continue to be a crucially important aspect of surface studies, the field of chemical reaction dynamics at surfaces is the real growth area in this field. The journal is well produced, and it will be interesting to see if it does emerge as a commercial success.

Interface Science is narrower in scope, being clearly aimed at the properties of solid–solid interfaces. The editor and his board are primarily drawn from the field of materials science. Apart from the journals described above, the main competitors are *Philosophical Magazine*, *Ultramicroscopy* and *Journal of Crystal Growth*. I believe that the publishers have marked

out an interesting subfield for development, and the first issues contain some interesting papers. The journal's success will depend critically on the response of the materials science community.

Tribology Letters is an imaginative and timely development. The important field of tribology — covering friction, lubrication and wear — is roughly at the stage of development that studies of the solid-gas interface had reached in 1964. The emphasis so far has been on the engineering aspects of these problems, based on measurements of macroscopic properties. But experimentalists and theoreticians are now beginning to make headway into the field at an atomic level. This journal specifically aims to bridge the gap between these two communities, and between them the editors (straddling the Atlantic, a smart move by the publisher) and their editorial board do span the two communities. This welcome, well-produced journal may well play a key role in bringing those working on new highly refined techniques at the atomic scale into contact with the tribological engineers, to the benefit of both. I hope so. □

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Material matters

Neil Alford

Advanced Performance Materials. Editor-in-chief F. H. Froes. *Kluwer*. 4/yr. Dfl358, \$205.50 (institutional); Dfl110, \$110 (personal).

Euromaterials. Managing editor Peter Gregory. *VCH*. 4/yr. DM150 (institutional); DM58 (personal).

It is always interesting to see how editors justify the introduction of a new journal. The intended niche for *Advanced Performance Materials* is the "transitioning [sic] of good science to real world applications". This is laudable, for making this transition is one of the most frustrating and difficult tasks in science and technology. The journal is aimed at both researchers and funding managers. According to the opening editorial, it "will not contain fundamental science papers, no matter how good" — which seems entirely at odds with one of the journal's stated aims of attracting original research papers. The big question, then, is what will the journal contain?

The answer is a broad spectrum of articles across a wide range of materials (metals, ceramics, polymers, composites, electronic materials) — but all oriented towards applications. There are broad

overviews of areas of science and technology (an article on structural ceramics, for example, contains no new or deep science but provides a good technological and economic appraisal); assessments of the materials research agenda in various countries; summaries of research at institutions such as the School of Mines in Madrid and La Sapienza in Rome; reports on specific materials such as interfacial reactions in Al/SiC composites; and whole issues devoted to accounts of work at organizations such as the All Russian Institute for Light Alloys (an issue is also planned for the Beijing Institute for Aeronautical Materials).

There is a stated emphasis on affordability of materials, particularly now that less and less research is geared towards the military. So-called 'dual use' technology can be used by the military as well as in the civilian arena. A good example is the infrared sensor. The military paid through the nose for this missile technology but it is now also used at very low cost in automatic doors and intruder alarms.

In an article on advanced-performance structural ceramics and ceramic composites, the most important reason for studying materials science is alluded to: the discovery of materials whose properties allow applications that would otherwise be impossible. If *Advanced Performance Materials* can help in the crucial problem of technology transfer, then it will succeed.

Already helping the development of materials science is *Euromaterials*, supplied free to individuals affiliated to the Federation of European Materials Societies (FEMS). A slender newsletter, it packs a lot in, from details about forthcoming conferences to reviews of recent books. But its main value is for the information it gives on funding opportunities, especially now that the European Union Framework programme is in full swing. With ECU1.6 billion (about £1.3 billion) on offer from Brite-Euram III over the next three years, it would seem a good idea to find out as much as possible about the programmes in Europe.

Each issue contains an 'Essay' of a couple of pages written by a leading materials scientist or by a 'Euro-official' responsible for coordinating funding in his or her country. Researchers can thus see where and how funds are likely to be allocated and how to gain access to them. A news section highlights the activities of FEMS in member countries, a useful source for those searching for European partners in cooperative research programmes. There are also sections on European news (essential reading for keeping abreast of, say, the Brite-Euram and NATO programmes) and on science and technology (equipment and materials). To round this all off, there are excellent and authoritative book reviews and an events calendar.

The European materials science com-

munity needs a newsletter of this sort and this is a good one. The importance of the discipline is reflected in the sizeable funds now devoted to materials science throughout Europe. *Euromaterials* will be of great help to those who want a slice of the cake. □

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Picking the combination

Kevin Kendall

Applied Composite Materials: An International Journal for the Science and Application of Composite Materials. Editor-in-chief Peter W. R. Beaumont. *Kluwer*. 6/yr. Dfl406, \$232.

COMBINE the word 'composite' with 'material', 'polymer', 'advanced' and 'technology' and you end up with around 16 possible journal titles, roughly the number today serving the multitude of material scientists, scientists and engineers working in this esoteric branch of technology.

Until now the study of composites has been as simple as the invention of journal titles. Take at least two materials, mix them together and eureka, you have a new composite material. For example, combinations of glass and polymer, carbon and resin, steel and cement, and ceramic and metal can all give beneficial properties. As scientists, we have even found the useless combinations worthy of interest in academic journals, especially when they are described as 'model systems'.

The introduction of the word 'applied' into a journal title changes all this. The message now is that the science has been over-fished. The combinations of materials requiring exploration have been over-exploited, and the myriads of micro-mechanisms have multiplied to the extent that historic fundamental theories seem to have been forgotten. It seems that engineers will replace scientists. Products will supersede prototypes.

In this respect, *Applied Composite Materials* does not quite live up to expectations. Writing papers containing applications as well as science is demanding. Only one paper out of 26 in the first four issues comes close to satisfying the criterion of describing application, measurement and theory. Its title is "Composite monocoque frame for a mountain bicycle: testing and calculation". The other papers are often interesting, typically 10–20 pages long, with a mix of microstructural, processing, testing and failure observations. But they could equally have appeared in

the existing *Journal of Composite Materials* or *Polymer Composites*.

The presentation of the papers is not pretentious. There are wide variations in the standard of writing, in the diagrams and in the style of references. Editorial comment is restrained, if not nonexistent. Refereeing, if any, does not seem to be stringent, and papers are accepted within a day in some cases, but within a month on average. But, then, what does one expect for US\$32 an issue?

The rapid acceptance of papers is perhaps due to the fact that most of them are written by the editors. With a board of 24 editors, it is eminently possible that no other authors are required. Having said that, the editors are of high standing in the composites community. A bright future for the journal is possible if good papers with applied emphasis can be attracted, especially if the number of authors from industry can be increased from the pathetically low showing in the first four issues. There is certainly a niche for a composites journal with an applied emphasis. □

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Free and essential

John Emsley

Main Group Chemistry News. Editor John Vassallo. *Gordon and Breach*. 4/yr. University libraries \$193, corporate libraries \$305; free to individuals.

Contemporary Organic Synthesis. Chairmen of editorial board G. Pattenden. *Royal Society of Chemistry*. 6/yr. Europe £165, USA \$303, elsewhere £173.

THERE is no reason for main-group inorganic chemists not to receive *Main Group Chemistry News* (MGCN), because it's free. All they have to do is contact the publishers, Gordon and Breach, and then four times a year they will receive a journal they can read while they eat their sandwiches. It will keep them well informed about the publisher's other journals and books targeted at main-group chemists. It also carries information about conferences, new products and the dates of forthcoming meetings.

Clearly, recipients of such a free sheet would soon start to discard it unopened, so something else is needed, something worth their reading. MGCN provides it in the form of 'overviews', of which there are between two and five per issue, some as long as 12 pages.

In the edition of June 1995 there are overviews on dendrimers, which are polymers with the structure of exotic blossoms, and phosphorus-31 nuclear magnetic resonance, which is an important analytical technique. Both are of sufficiently high academic

standard for the recipient of MGCN not only to read them but also to retain the issues for future reference. The only justification for a university librarian to subscribe to MGCN would be to build up a complete set of these useful overviews — assuming there is a main-group chemist or two on campus.

On the other hand, almost every campus has organic chemists, so libraries will have to subscribe to *Contemporary Organic Synthesis* because almost every organic chemist researches organic synthesis. Chemists are finding that they can, given a lot of skill and a little cunning, synthesize even the most exotic molecules found in nature. Each issue of *Contemporary Organic Synthesis* carries four lengthy articles in which chemists in a particularly active field of organic chemistry review the recent literature. Such articles will be very useful to those starting a PhD or to researchers wanting a quick way into an area. Each review is about 15 pages long and covers about 150 original papers. The journal also has reviews of the more normal kind, where the focus is narrower. For example, in the issue of February 1995, Alexander Oxford, of Glaxo-Wellcome, deals with migraine, the role of serotonin and the use of sumatriptan to control it.

Each paper in *Contemporary Organic Synthesis* is headed by a list of subheadings, and there are plenty of clearly drawn molecular structures and reaction sequences. The overall impression is of a journal whose authors have been well briefed, and which is produced with care and attention to detail. □

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Academic wasteland

Jane Powell

Journal of Waste Management and Resource Recovery. Editor P. C. Coggins. EPP. 4/yr. £49, \$59.

I GENERALLY find journals on waste management disappointing. Although I am sure that papers on "the oxidation of organic compounds in waste" are fascinating to some, for many they are of marginal interest. So I eagerly awaited the arrival of this new journal. With Chris Coggins as editor, a person certainly inclined towards policy issues, I looked forward not only to a good read but also to the discovery of a possible destination for my own research papers.

The first number begins not with an

engaging leading article or welcoming address, but instead has something called 'Notes', an interesting enough review of current waste-management news that would have found a better home at the end of the issue. The papers, however, do not disappoint, covering a satisfactory mix of recycling issues, hazardous household waste collections, incineration economics, waste-management strategies and life-cycle assessment — inevitably rather predictable subjects for a new journal. In subsequent issues there is an emphasis on waste strategy and the upper end of the waste hierarchy (recycling for example), although papers on other disposal options are invited. (A challenge to the waste hierarchy would make for a refreshing read). The authors, however, are mainly consultants, or researchers primarily involved in consultancy. Where are the university researchers? Is waste-management research still not considered an academic subject?

The journal unquestionably fills a gap in the waste-management literature. The quality of production is good and we are told that papers received are reviewed promptly. But it needs to attract more academics if it is to establish itself as anything more than a promotional outlet for consultants. □

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Weather report

Jean Palutikof

Meteorological Applications. Editor R. W. Riddaway. *Royal Meteorological Society*. 4/yr. USA, Canada and Mexico \$172, elsewhere £96.

IN the leaner and fitter economies of the 1980s and 1990s, the savings to be achieved by appropriate exploitation of meteorological data are increasingly appreciated. A substantial community of researchers has emerged whose goal is to analyse these data for specific applications. Examples that come readily to mind include forecasting for ice formation on roads (to establish whether salting is necessary) and for the retail sector (to control the flow of goods to the shops).

Until the appearance of *Meteorological Applications*, this research community had no obvious forum for the presentation of results, particularly in Europe. The stated aim of the journal is to serve the complete range of interests within weather services: that is, according to the opening editorial in the inaugural issue, "all applied meteorologists, forecasters and users of

meteorological services".

The journal is firmly based in the European context. Of the 40 articles in the first volume, only three originate outside Europe, and all but eight are concerned with regions inside the continent. Given that North American counterpart journals exist, the decision to focus on Europe is wise. It also allows scope to fulfil a need within the European research community: following the example of *Bulletin of the American Meteorological Society*, news of forthcoming meetings, calls for papers and conference reports are published, along with letters and book reviews.

By comparison with other journals in the field of meteorology, the papers are short. But because some (perhaps all) papers in the first two numbers are based on presentations at the First European Conference on Applications of Meteorology, it is perhaps too early to judge quantity and quality. Production standards are high and several papers include colour illustrations. The price is typical for a journal of this kind.

There is some overlap with existing journals. Particularly in the later numbers of the first volume, papers appear on 'downscaling' from coarse-resolution climate models, and on mesoscale and synoptic events. These do not really fall within the area of applied meteorology. The risk is twofold: that these papers may be overlooked in a literature search and that the journal will become just another outlet for papers on standard meteorological topics. Given that there is a clear niche to fill, it would be a pity to dilute the content in this way. Provided that the journal is faithful to its stated aims, it should fulfil a real need and find a ready market. □

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The unpredictable Earth

David Bercovici

Nonlinear Processes in Geophysics. Managing editor Arne K. Richter. *European Geophysical Society*. 4/yr. DM198 \$130 (institutional); DM90, \$60 (members).

At the risk of offending (as many) particle physicists (as possible), I will begin with the bold contention that there is no more universal or fundamental science than the study of nonlinear and complex systems. It has countless applications in, for example, climate variations, plate tectonics, population dynamics, weather prediction, cardiac fibrillation, earthquakes, stock prices and

Journals also submitted for review

The following is a list of journals received that were eligible for review but which for one reason or another are not covered in the supplement. The list does not include journals sent in that had not published enough titles to be considered.

Automated Software Engineering (Kluwer)
Chemical Technology Europe (VCH)
Clinical and Diagnostic Laboratory Immunology (American Society for Microbiology)
Energy for Sustainable Development (International Energy Initiative)
Engineering Failure Analysis (Pergamon)
European Journal of Plant Pathology (Kluwer)
The Global Atmosphere and Ocean System (Gordon and Breach)
International Journal of Microcirculation (Karger)
Journal of Endotoxin Research (Churchill Livingstone)

Journal of Enhanced Heat Transfer (Gordon and Breach)
Microcirculation: The Official Journal of the Microcirculatory Society, Inc. (Chapman and Hall)
Psychology Crime and Law (Harwood Academic)
Reliability, Quality and Safety Engineering (World Scientific)
TB and HIV Quarterly (Sid Alerte Internationale)
Transgenics: Biological Analysis Through DNA Transfer (Harwood Academic)
VLSI Design: An International Journal of Custom-Chip Design, Simulation, and Testing (Gordon and Breach)
Wavelength: The Journal of Science Society and the Media (University of the West of England)
International Journal of Uncertainty, Fuzziness and Knowledge-Based Systems (World Scientific)

probably the formation of life itself.

Nonlinear processes are seen in the dynamics of the Earth's many spheres, from the magnetosphere to the atmosphere and from the ocean to the Earth's core (which, to complete the circle, generates the magnetosphere). So the launch of *Nonlinear Processes in Geophysics* is certainly timely and appropriate.

But can a field as interdisciplinary as nonlinear science still be interdisciplinary if it concentrates on the Earth sciences? The answer is an unequivocal 'yes'. The geosciences are essentially founded on the application of mathematics, physics, chemistry and biology to our natural environment. *Nonlinear Processes in Geophysics* largely succeeds in treating these basic fields with as much variety as in a comparable but more general journal such as *Nonlinearity*. The journal's offerings so far cover plankton population dynamics, climate variations, oceanic, atmospheric and geophysical fluid dynamics, analysis of radiocarbon dating, earthquakes, topography, the magnetosphere and rainfall. But even with this impressive list of topics, the equitable representation of disciplines still needs some work. Nearly two-thirds of the contributions deal with the atmosphere and ocean. Although the first article in the journal concerns plankton dynamics, it is so far the only paper with a biological twist, a likely consequence of the use of the word 'geophysics' in the journal's title instead of 'geosciences'. But some classical 'geophysical' fields (namely those involving everything beneath the Earth's surface, such as magma and melt processes, mantle convection and especially the core dynamo) are conspicuously absent.

Nevertheless, the journal shows great potential for offering a truly interdisciplinary

approach. It allows researchers and students alike to recognize themes common to different articles, such as concepts of multifractal analysis and self-organized criticality applied to atmospheric turbulence, earthquakes, landslides, rainfall and geomorphology.

The overall scientific quality and clarity of the papers are reasonably high, although some of the contributors could have been encouraged to write for a broader audience than just their colleagues. More review articles (I counted only one) would help to foster such interdisciplinary communication and comprehension.

The journal offers good value for both subscribers and contributors (there are no page charges); production quality is reasonable and the layout is fairly uniform in appearance; and time from submission to acceptance of papers is a fairly average 6–9 months.

Scientists (like other humans) enjoy a fine whine, and one of their favourite complaints is about the proliferation of journals. No one denies that the excessive number of articles and journals prohibits busy scientists from keeping abreast of the literature. But it is important for scientists to experiment with as many different combinations and permutations of disciplines as possible. New journals provide an important platform for such experimentation. In offering fresh approaches, *Nonlinear Processes in Geophysics* is set on a promising path. □

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